SOMATOSTATIN AND SUBSTANCE P ANALOGUES:

APPLICATIONS IN AUTOIMMUNE AND HAEMATOLOGICAL DISEASES

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SOMATOSTATIN AND SUBSTANCE P ANALOGUES:

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Somatostatine en substance P analoga:
toepassingen in autoimmuun- en haematologische ziekten

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In memory of Huib and my father

CONTENTS

PUBLICATIONS BASED ON THE STUDIES DESCRIBED IN THIS THESIS

Chapter 2 van Hagen PM, Krenning EP, Kwekkeboom DJ, Reubi JC, van den Anker-Lugtenburg PJ, Löwenberg B, Lamberts SWJ. Somatostatin and the immune and haematopoietic system; a review. [Review] Eur J Clin Invest 1994;24:91-9.

Chapter 3.1 Reubi JC, Waser B, van Hagen PM, Lamberts SWJ, Krenning EP, Gebbers JO, Laissue JA. In vitro and in vivo detection of somatostatin receptors in human malignant lymphomas. Int J Cancer 1992;50:895-900.

Chapter 3.2 van Hagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY, Löwenberg B, Lamberts SWJ. Somatostatin analogue scintigraphy of malignant lymphomas. Br J Haematol 1993;83:75-9.

Chapter 3.3 van den Anker-Lugtenburg PJ, Krenning EP, Oei HY, Gerrits CJH, van Hagen PM, Reubi JC, Lamberts SWJ, Löwenberg B. The role of somatostatin receptor scintigraphy (SRS) in the initial staging of malignant lymphoma. [Abstract] J Nucl Med 1994;35:132.

van Hagen PM, van den Anker-Lugtenburg PJ, Reubi JC, Lamberts SWJ, Krenning EP. The role of octreotide scintigraphy in malignant lymphomas, granulomatous diseases and rheumatoid arthritis. Handbook of targeted delivery of imaging agents. Torchilin VP, editor. CRC Press, Inc., 1995:631-44.

Chapter 4.2 van Hagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue JA, Hoogsteden HC, Lamberts SWJ. Somatostatin analogue scintigraphy in granulomatous diseases. Eur J Nucl Med 1994;21:497-502.

Chapter 5.1 van Hagen PM, Markusse HM, Lamberts SWJ, Kwekkeboom DJ, Reubi JC, Krenning EP. Somatostatin receptor imaging. The presence of somatostatin receptors in rheumatoid arthritis. Arthritis Rheum 1994;37:1521-7.

Chapter 5.2 Reubi JC, Waser B, Markusse HM, Krenning EP, van Hagen PM, Laissue JA. Vascular somatostatin receptors in synovium from patients with rheumatoid arthritis. Eur J Pharmacol 1994;271:371-8.

Chapter 6 van Hagen PM, Lichtenauer-Kaligis EGR, ten Bokum AMC, Visser-Wisselaar HA, Hofland LJ, Hazenberg MP, van Dongen JJM, Lamberts SWJ. Somatostatin receptor subtype expression in human lymphoid cell lines. Submitted for publication.

Chapter 8.1 Breeman WAP, van Hagen PM, Visser-Wisselaar HA, van der Pluijm ME, Koper JW, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Lamberts SWJ, Visser TJ, Krenning EP. Substance P receptor scintigraphy: initial studies in vitro and in rats. J Nucl Med, in press.

Chapter 8.2 van Hagen PM, Breeman WAP, Reubi JC, Postema PTE, van den Anker-Lugtenburg PJ, Robers C, Lamberts SWJ, Kwekkeboom DJ, Visser TJ, Krenning EP. Thymus scintigraphy with [^{111}In-DTPA-Arg^{1}]-substance P. Submitted for publication.

AIM OF THE STUDY

Aim of the study

Somatostatin has been extensively studied in relation to the endocrine and nervous systems. Many reports on the role of somatostatin receptor imaging and somatostatin treatment of neuroendocrine tumours have been published. The relation between somatostatin and other neuropeptides and the immune system is less explored.

The aim of this study was to investigate the diagnostic applications of somatostatin and substance P analogues in autoimmune and haematological diseases. Both in vivo and in vitro studies were performed, using peptide receptor scintigraphy in patients and rats, peptide receptor autoradiography on tissue biopsies, ligand binding assays on cell homogenates, and polymerase chain reactions on lymphoid cell lines. Moreover, based on the results of these studies, speculations were made about the therapeutical applications of somatostatin and substance P analogues in autoimmune and haematological diseases.

CHAPTER 1

AN INTRODUCTION TO SOMATOSTATIN AND SOMATOSTATIN RECEPTOR SCINTIGRAPHY

Somatostatin

Somatostatin belongs to the expanding family of small peptides that are characterized by a variety of actions in different organ systems throughout the body (1,2). Somatostatin was originally detected as an inhibitor of the release of growth hormone by the pituitary gland, and was initially called 'somatotrophin-release inhibiting factor'. It was subsequently identified as a cyclic peptide consisting of 14 amino acids (somatostatin 14) with a disulphide bridge connecting the two cysteine residues (Figure 1) (3,4). A larger precursor molecule was identified as somatostatin 28, or prosomatostatin (Figure 1) (1,2). Preprosomatostatins are even larger peptides with somatostatin 28 located at the C terminus (5). These larger molecules are spliced into the smaller somatostatin 14 by a processing enzyme (6). Somatostatin is found throughout the human body, but mainly in the endocrine glands and nervous system (1,2). In Table 1 the anatomical distribution of somatostatin in humans is shown.

Somatostatin receptors

Schönbrunn et al. first described somatostatin receptors on the rat pituitary tumour cell line GH4C1 (7). Ligand binding assays on tissue homogenates and autoradiography of tissue sections, using [^{125}I-Tyr^{3}]-octreotide, subsequently identified somatostatin receptors on a wide range of tumours and inflammatory tissues (8,9). The highest density of high affinity receptors were found on tumours with characteristics of the amine precursor uptake and decarboxylation (APUD) system, like paragangliomas (10-11).

Five subtypes of human somatostatin receptors have been identified, all with a high affinity for somatostatin 14 (IC_{50} = 1.1-2.1 nM) and somatostatin 28 (IC_{50} = 0.25-5.4 nM) (12). For somatostatin 14 the following order of increasing affinity was found: subtype 3, subtype 5, subtype 2, subtype 1, subtype 4. The affinity of receptor subtypes 1-4 was two- to fourfold higher for somatostatin 14 than for somatostatin 28, whereas receptor subtype 5 bound somatostatin 28 with eightfold higher affinity (12). Characteristics of the human somatostatin receptors are shown in Table 2.

Somatostatin 14

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

Somatostatin 28

Ala - Gly - Cys - Lys - Asn - Phe - Phe - Trp - Lys - Thr - Phe - Thr - Ser - Cys
|
Lys - Arg - Glu - Arg - Pro - Ala - Met - Ala - Pro - Asn - Ser - Asn - Ala - Ser

Octreotide

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

[^{125}I-Tyr^{3}]-octreotide

D-Phe - Cys - Tyr - D-Trp - Lys - Thr - Cys - Thr(ol)
|
^{125}I

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

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

BIM-23014

D-Nal - Cys - Tyr - D-Trp - Lys - Val - Cys - Thr(ol)

RC-160

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

Figure 1. Sequence of somatostatin and some somatostatin analogues.

Somatostatin receptors are coupled to G proteins in the cell membrane, and generate a transmembrane signal after binding of somatostatin. Three receptor linked effector systems have been identified. Binding of somatostatin to the receptor leads to a reduction in the intracellular level of cyclic adenosine monophosphate (a) (13,14), and ionized calcium (b) (15,16), and an increase in the tyrosine phosphatase activity (c) (17).

Table 1. Anatomical distribution and actions of somatostatin in humans (1).

Localization	Action
Central nervous system (cortex, limbic system, brain stem, and spinal cord)	Neurotransmitter
Anterior pituitary gland	Neurohormone
Peripheral nervous system (sensory neurons)	Neurotransmitter
Endocrine glands	Inhibitor of hormone- and (neuro)peptide secretion (gastrin, pancreatic polypeptide, vasoactive intestinal peptide, insulin, glucagon, and substance P)
Exocrine glands	Inhibitor of gastric acid- and salivary fluid secretion Regulator of intestinal epithelial electrolyte- and water release, and of pancreatic enzyme release.
Gastrointestinal tract	Inhibitor of motor activity of stomach, small intestine and gall bladder
Vascular system	Regulator of coeliac-, mesenteric- and renal blood flow

Clinical applications of somatostatin

The general inhibitory effect of somatostatin on hormone secretion of various glands led to the concept of a possible beneficial effect of somatostatin analogues in the treatment of patients with diseases based on an overproduction of hormones by endocrine active tumours. The short half-life of somatostatin 14, however, makes it unsuitable for routine treatment. Therefore, various somatostatin analogues have been developed for therapeutic purposes. The somatostatin analogue octreotide (Sandostatin®) was initially developed for the inhibition of gastric acid secretion. However, the required parenteral administration made the drug less suitable when compared with the H-2 receptor antagonists. In contrast to somatostatin 14, the half-life of octreotide is 90-120 minutes when administered subcutaneously. The pharmacodynamic effect lasts even for 8-12 hours (18). Octreotide has only few and infrequent side effects; cholelithiasis during

Table 2. Characteristics of human somatostatin receptor subtypes 1-5 (12).

	Somatostatin receptor subtype				
	1	2	3	4	5
Chromosomal location	14	17	22	20	16
Number of amino acids	391	369	418	388	363
G protein coupling	+	+	+	+	+
K^+/Ca^{2+} channels	-	+	-	-	-
Adenylyl cyclase coupling	+	+	+	+	+
Selectivity of somatostatin analogues					
Somatostatin 14	+	+	+	+	+
Somatostatin 28	+	+	+	+	+
BIM-23014	-	+	+/-	-	+
RC-160	-	+	-	-	+
Octreotide	-	+	+/-	-	+

chronic treatment, hyperglycaemia, and ileus with nausea and vomiting (19,20). Based on its inhibiting properties, octreotide is now tested in other diseases like pancreatitis, gut motility disorders, upper gastrointestinal haemorrhage (21-28), and endocrine active tumours. The application of the antiproliferative properties of octreotide in oncology is under current investigation (29,30).

The effects of octreotide were found to be mediated mainly through somatostatin receptor subtypes 2 and 5 (12) (see Table 2). Other cyclic somatostatin analogues, like BIM-23014 and RC-160 (see Figure 1 and Table 2), were also studied for their possible clinical application (30). New subtype specific somatostatin analogues and antagonists are currently being developed in order to study the precise function of the somatostatin receptor subtypes. Moreover, subtype specific somatostatin analogues may be of particular interest for clinical application.

Somatostatin receptor scintigraphy

The concept of peptide receptor scintigraphy is simple. After intravenous injection, radioactive and non-radioactive peptides (ligands) bind to cells that have membrane binding sites for these ligands. One mechanism by which ligands can enter the cells is endocytosis of ligand-receptor complexes. This occurs after binding of the ligand to the receptor, and clustering of the complexes in the plasma membrane. In the cell, metabolism of the radioligand takes place with recycling of the receptor after dissociation from the ligand. Another route of cellular entry is transport over the plasma membrane by a carrier mechanism. If the density of high affinity receptors on cells, or the number of cells with high affinity receptors, in a specific tissue is higher than in the surrounding tissue, the radioactive signal from this tissue will be higher than from the surrounding tissue. A gamma camera is used to display the radioactive signal.

Somatostatin receptor positive human neuroendocrine tumours can be visualized using radioiodinated analogues of somatostatin (31,32). However, these pharmaceutical products have some major drawbacks like the hepato-biliary clearance and subsequent faecal excretion (32). Like radioiodinated somatostatin analogues, [^{111}In-DTPA-D-Phe^{1}]-octreotide binds specifically to somatostatin receptors (33). Some remarkable advantages of this radioligand over iodinated somatostatin analogues are its easy preparation, its appropriate half-life, and its mainly renal clearance. Faecal excretion of radioactivity amounts to only a few percent of the administered dose (33,34). Therefore, [^{111}In-DTPA-D-Phe ^{1}]-octreotide is highly suitable for its use in planar imaging and single photon emission computed tomography (SPECT) of the abdomen, which is of particular importance in the localization of small neuroendocrine tumours in this part of the body.

After administration of [^{111}In-DTPA-D-Phe ^{1}]-octreotide, physiological accumulation of radioactivity is found in the kidneys, spleen, urinary bladder, liver, pituitary gland, thyroid gland, and salivary glands. Other sites of deposition of radioactivity are considered to be pathological (34). The excretion of [^{111}In-DTPA-D-Phe ^{1}]-octreotide, occurring mainly via the kidneys, results in 90% of the administered dose being present in the urine 24 hours after injection. Because of its relatively long effective half-life, [^{111}In-DTPA-D-Phe^{1}]-octreotide can be used to efficiently visualize somatostatin receptor

bearing tumours and inflammatory tissue 24 and 48 hours after intravenous injection, at which time the interfering background radioactivity is minimized by renal clearance of the radiopharmaceutical (33-35). Moreover, it can be used to irradiate tumours which express a high density of somatostatin receptors, since it emits Auger- and conversion electrons (36).

In our scintigraphic studies 200-250 MBq of [^{111}In-DTPA-D-Phe^1]-octreotide is injected intravenously. Planar images are obtained with a gamma camera. Preset counts for images obtained 24 hours after injection of the [^{111}In-DTPA-D-Phe^1]-octreotide are 300,000 per spotview for the head and neck, and 500,000 per spotview for the remainder of the body. The preset counting time for images obtained 48 hours after injection of the [^{111}In-DTPA-D-Phe^1]-octreotide is 15 minutes (37). Images are divided in parts that are easily recognized on scintigrams, i.e. head and neck, chest, upper abdomen, lower abdomen (division line: lower edges of the kidneys), and the limbs.

References

1. Reichlin S. Somatostatin (first of two parts). N Engl J Med 1983;309:1495-501.
2. Reichlin S. Somatostatin (second of two parts). N Engl J Med 1983;309:1556-63.
3. Krulich I, Dhariwal AP, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology 1968;83:783-90.
4. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 1973;179:77-9.
5. Lucey MR. Endogenous somatostatin and the gut. Gut 1986;27:457-67.
6. Mehler PS, Sussman AL, Maman A, Leitner JW, Sussman KE. Role of insulin secretagogues in the regulation of somatostatin binding by isolated rat islets. J Clin Invest 1980;66:1334-8.
7. Schönbrunn A, Tashjian H Jr. Characterization of functional receptors for somatostatin in rat pituitary cells in culture. J Biol Chem 1978;253:6473-83.
8. Reubi JC, Krenning EP, Lamberts SWJ, Kvols LK. Somatostatin receptors in malignant tissues. J Steroid Biochem Mol Biol 1990;37:1073-7.
9. Reubi JC, Kvols LK, Krenning EP, Lamberts SWJ. Distribution of somatostatin receptors in normal and tumor tissue. Metabolism 1990;39 Suppl 2:78-81.
10. Reubi JC, Maurer R, von Werder K, Torhorst J, Klijn JGM, Lamberts SWJ. Somatostatin receptors in human endocrine tumors. Cancer Res 1987;47:551-8.
11. Sagman U, Mullen JB, Kovacs K, Kerbel R, Ginsberg R, Reubi JC. Identification of somatostatin receptors in human small cell lung carcinoma. Cancer 1990;66:2129-33.

12. Patel YC, Greenwood MT, Warszynska A, Panetta R, Srikant CB. All five cloned human somatostatin receptors (hSSTR1-5) are functionally coupled to adenylyl cyclase. Biochem Biophys Res Commun 1994;198:605-12.
13. Koch BD, Blalock JB, Schönbrunn A. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. I. J Biol Chem 1988;263:216-25.
14. Koch BD, Schönbrunn A. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. II. An increase in potassium conductance initiates somatostatin-induced inhibition of prolactin secretion. J Biol Chem 1988;263:226-34.
15. Lewis DL, Weight FF, Luini A. A guanine nucleotide-binding protein mediates the inhibition of voltage-dependent calcium current by somatostatin in a pituitary cell line. Proc Natl Acad Sci U S A 1986;83:9035-9.
16. Ikeda SR, Schofield GG. Somatostatin blocks a calcium current in rat sympathetic ganglion neurones. J Physiol 1989;409:221-40.
17. Liebow C, Reilly C, Serrano M, Schally AV. Somatostatin analogues inhibit growth of pancreatic cancer by stimulating tyrosine phosphatase. Proc Natl Acad Sci U S A 1989;86:2003-7.
18. Pless J. Chemistry and pharmacology of Sandostatin®. Z Gastroenterol 1990;28 Suppl 2:4-7.
19. Lembcke B, Creutzfeldt W, Schleser S, Ebert R, Shaw C, Koop I. Effect of the somatostatin analogue Sandostatin (SMS 201-995) on gastrointestinal, pancreatic and biliary function and hormone release in normal men. Digestion 1987;36:108-24.
20. Christensen SE, Weeke J, Orskov H, Kaal A, Lund E, Jorgensen J, Harris AG. Long-term efficacy and tolerability of octreotide treatment in acromegaly. Metabolism 1992;41:44-50.
21. Bauer H, Dienicke A, Holle R. Prevention and treatment of upper gastrointestinal haemorrhage with cimetidine and somatostatin in intensive care patients. Anaesthesist 1977;26:662-4.
22. Kayasseh L, Gyr K, Stalder GA, Allgöwer M. Somatostatin in acute gastroduodenal haemorrhage. Lancet 1978;2:833-4.
23. Thulin L, Tyden G, Samnegard H, Muhrbeck O, Efendic S. Treatment of bleeding oesophageal varices with somatostatin. Acta Chir Scand 1979;145:395-8.
24. Kayasseh L, Gyr K, Keller U, Stalder GA, Wall M. Somatostatin and cimetidine in peptic-ulcer haemorrhage. A randomised controlled trial. Lancet 1980;1:844-6.
25. Limberg B, Kommerell B. Somatostatin for cimetidine-resistant gastroduodenal haemorrhage. Lancet 1980;2:916-7.
26. Basso N, Bagarini M, Quondamcarlo C, Albertini V, Ziparo V, Anza M, Mari F, Procacciante F, Grassini G, Percoco M, Marocco T, Cucchiara, Bracci M. Effective control of variceal bleeding by somatostatin: A double blind randomized crossover study [abstract]. Gastroenterology 1983;84:1100.
27. Jenkins SA, Baxter JN, Corbet WA, Devitt P, Ware J, Shields R. A prospective randomised controlled clinical trial comparing somatostatin and vasopressin in controling acute variceal haemorrhage. Br Med J 1985;290:275-8.
28. Usadel KH, Kessler H, Rohr G, Kusterer K, Palitzsch KD, Schwedes U. Cytoprotective properties of somatostatins. Klin Wochenschr 1986;64 Suppl 7:59-63.
29. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. Endocr Rev 1991;12:450-82.

30. Weckbecker G, Raulf F, Stolz B, Bruns C. Somatostatin analogs for diagnosis and treatment of cancer. Pharmacol Ther 1993;60:245-64.
31. Krenning EP, Bakker WH, Breeman WAP, Koper JW, Kooij PPM, Ausema L, Lameris JS, Reubi JC, Lamberts SWJ. Localization of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989;1:242-4.
32. Bakker WH, Krenning EP, Breeman WAP, Kooij PPM, Reubi JC, Koper JW, de Jong M, Lameris JS, Visser TJ, Lamberts SWJ. In vivo use of a radioiodinated somatostatin analogue: dynamics, metabolism, and binding to somatostatin receptor-positive tumors in man. J Nucl Med 1991;32:1184-9.
33. Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyono-Han B, de Jong M, Kooij PPM, Bruns C, van Hagen PM, Marbach P, Visser TJ, Lamberts SWJ. In vivo application of [^{111}In-DTPA-D-Phe ^{1}]-octreotide for detection of somatostatin receptor-positive tumors in rats. Life Sci 1991;49:1593-601.
34. Krenning EP, Bakker WH, Kooij PPM, Breeman WAP, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwekkeboom DJ, Reijs AEM, van Hagen PM, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe ^{1}]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^{3}]-octreotide. J Nucl Med 1992;33:652-8.
35. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.
36. Krenning EP, Kooij PPM, Bakker WH, Breeman WAP, Postema PTE, Kwekkeboom DJ, Oei HY, de Jong M, Visser TJ, Reijs AE, Lamberts SWJ. Radiotherapy with a radiolabeled somatostatin analogue, [^{111}In-DTPA-D-Phe ^{1}]-octreotide. A case history. Ann N Y Acad Sci 1994;733:496-506.
37. Krenning EP, Bakker WH, Kooij PPM, Breeman WAP, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwekkeboom DJ, Reijs AEM, van Hagen PM, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with indium-111-DTPA-D-Phe-1-octreotide in man: metabolism, dosimetry and comparison with iodine-123-Tyr-3-octreotide. J Nucl Med 1992;33:652-8.

CHAPTER 2

SOMATOSTATIN AND THE IMMUNE AND HAEMATOPOIETIC SYSTEM

Introduction

Neuroimmunoendocrinology is a rapidly evolving field of research which until recently has had limited clinical applications. The interactions between (neuro)peptides and immune competent cells have been investigated in a wide variety of in vitro culture systems and animal models. The current knowledge in this field has been summarized in a number of excellent reviews (1-4). Our own interests stem mainly from our clinical observations on the value of somatostatin receptor visualization in a number of immune disorders. We originally developed this technique, in which an isotope coupled somatostatin analogue is injected, in order to localize primary endocrine tumours such as medullary thyroid cancers, small cell lung cancers, islet cell tumours and carcinoids, and in addition their metastases.

In the present review we summarize the knowledge on somatostatin receptor expression in lymphoid tissue, and on the significance of this peptide in the modulation of immune functions.

Somatostatin

Somatostatin was initially described as a growth hormone-release inhibiting factor synthesized in the hypothalamus (5,6). However, somatostatin operates also as a neurotransmitter in the brain, as an inhibitor of gut motility, and as an important regulatory peptide for the release of several peptide hormones, including gastrin, insulin, glucagon and cholecystokinin (5,6). Somatostatin is composed of 14 or 28 amino acids. The 14 amino acid structure predominates in all tissues with the exception of the duodenum and ileum. Because of the very short plasma half-life (several minutes) of native somatostatin, a number of long acting somatostatin analogues, like octreotide (Sandostatin®), have been developed for therapeutic purposes (7,8). Octreotide is clinically used in the control of hormonal hypersecretion in patients with acromegaly, islet cell tumours and carcinoids, but preliminary data also suggest its effectiveness in variceal bleeding, acute and chronic pancreatitis, dumping syndrome and cancer therapy (9,10).

Somatostatin receptors on lymphoid cells, monocytes and cell lines

Specific receptors for somatostatin, mediating the various actions of this peptide, have been described in normal as well as tumour tissues (11). Bhathena et al. first identified receptors on human mononuclear leucocytes (12). Specific low affinity receptors were found on resting monocytes and lymphocytes. Monocytes expressed 450-500 receptors per cell as compared with 220-250 receptors per cell in the lymphocyte fraction. Subsequently, somatostatin receptors were identified on more than 95% of normal mitogen activated human peripheral lymphocytes, using a binding assay (13). Resting peripheral blood lymphocytes, granulocytes and red blood cells did not present somatostatin receptors. The activated lymphocytes expressed a single class of low affinity receptors (K_d = 10.8 nM). No distinct predilection of somatostatin receptor positive cells among lymphocyte subpopulations was found.

Using ^{125}I labelled somatostatin and fluorescent labelled somatostatin, approximately 50% of murine B and T lymphocytes prepared from Peyer's patches and 30% of lymphocytes from the spleen were shown to express somatostatin receptors (14). In the Peyer's patches T and B lymphocytes bound fluorescent somatostatin regardless of the T cell surface antigen (pan T cells 84%, T helper cells 79%, T suppressor cells 94%) or surface immunoglobulin isotype. However, in lymphocytes obtained from the spleen approximately 30% of pan T and T helper cells, 52% of T suppressor cells, and especially the IgA membrane positive B lymphocytes were shown to bind fluorescent somatostatin (14). In addition, 87.5% of the primary human lymphoblastic leukaemias express somatostatin binding sites of two different affinities, compared with 12.5% of the non-lymphocytic leukaemias (13). A Jurkat line of human leukaemic T cells and the U-266 IgE producing myeloma cells also bind fluorescent labelled and radiolabelled somatostatin (15). Scatchard analysis of this competitive binding indicated high and low affinity binding sites on both cell lines. The human adult T cell leukaemia cell line MT-2, the human T cell line Molt-4F, and the Epstein-Barr virus transformed B cell line Isk were investigated by Nakamura et al. (16). The MT-2 and Isk cell lines showed large numbers of high affinity somatostatin receptors (K_d = 0.64 nM and K_d = 1.1 nM, respectively), while the Molt-4F cell line possessed less receptors. No receptors were found on the Jurkat cell line, which is in contrast with the observations by Sreedharan et al. (15).

Summarizing, somatostatin receptors are expressed on normal as well as activated monocytes, lymphocytes, lymphocytic leukaemic cells and lymphoid cell lines. Normal granulocytes and red blood cells do not possess somatostatin receptors. Normal lymphocytes have low affinity receptors, activated lymphocytes and leukaemic cells present somatostatin receptors with K_d values in the nanomolar range, which suggests that somatostatin receptor expression is related to activation and/or proliferation of these cells. The greater proportion of somatostatin receptor positive cells in Peyer's patches may be related to continuous antigenic stimulation. The expression of two types of somatostatin receptors in human lymphocytic leukaemic cells may be the result of malignant transformation. These studies further suggest that somatostatin receptors are expressed in cells that originate from the lymphocytic and monocytic system without a distinct predilection for lymphocyte subsets.

Somatostatin and somatostatin receptors on lymphoid tissue and the role of the peripheral nervous system

Somatostatin has been identified in all brain regions, the highest levels being present in the hypothalamus, in the limbic and mesencephalic regions and in the basal ganglia (17,18). Somatostatin is also found in peripheral tissues, like the endocrine glands, the gastrointestinal system and the peripheral nervous system. In the peripheral nervous system, somatostatin is found in C fibres and the small myelinated A-delta fibres. The peptide occurs in the synaptosomal fraction of axon terminals and is released in vitro upon electrical depolarization in the presence of calcium. The somatostatin content of primary afferent nerves is not related to the functional differences in sensory function, which suggests that somatostatin may serve as a local modulator. It has been suggested that local immune responses may be mediated by neuropeptides like somatostatin, which is released into the microenvironment by sensory nerves (19).

Lymphoid organs contain autonomic nerve fibres, which are associated with blood vessels, lymphocytes, and macrophages. The presence of neurotransmitters in these fibres, directly innervating the parenchymal elements of lymphoid organs, establishes an anatomical link with the nervous system, which provides a route for the translation of central nervous system

activity into signals that can influence the functions of specific cells in the immune system. Noradrenergic innervation of lymphoid tissue is particularly related to the zones of T lymphocytes and thymocytes (20). Somatostatin receptors have been detected in mouse spleen and Peyer's patches (14). Canine mesenteric lymph nodes express calcitonin gene related peptide and substance P receptors but no receptors for substance K, somatostatin and bombesin (21). In contrast, human gut associated lymphoid tissues (palatine tonsils, ileal Peyer's patches, vermiform appendix, and colonic solitary lymphatic follicles) present somatostatin receptors as detected by in vitro receptor autoradiography using [^{125}I-Tyr^{3}]-octreotide, somatostatin 14 and somatostatin 28 (22). These receptors are preferentially located in the germinal centres. The luminal parts of the centres are more strongly labelled than the basal parts. This observation suggests that the expression of somatostatin receptors is related to the proliferative state of lymphoid cells, and that the action of somatostatin takes place in activated lymphoid tissue. Germinal centres are sites of intensive B cell lymphoblast proliferation. The receptors found in these lymphoid tissues probably correspond best to a somatostatin receptor which binds with high affinity to somatostatin 14, somatostatin 28, as well as octreotide (22).

Messenger ribonucleic acid (mRNA) encoding for somatostatin has been found in the spleen and thymus of male rats, and in the spleen, thymus and bursa of Fabricius of chickens (23,24). The somatostatin concentration in rat thymus was higher than in rat spleen. The somatostatin concentration in the bursa of Fabricius of the chicken was also higher than in the spleen. In the spleen the somatostatin positive cells were located especially in clusters in the white pulp, and more dispersed in the red pulp. The somatostatin positive cells in the thymus were located in the medulla and around the corticomedullary junction. Large clusters of positive cells were observed in the medullary portion of the nodules of the bursa of Fabricius. In the rat, dispersed spleen B lymphocytes were somatostatin positive, while in the thymus a small population of thymocytes contained somatostatin (23).

Summarizing, peptidergic neurons may contain somatostatin, while somatostatin receptors can be found in normal lymphoid tissue. The anatomical link between the nervous system and lymphoid tissue suggests that the nervous system may directly modulate the immune system via somatostatin receptors. In addition, the presence of somatostatin mRNA in

lymphoid tissue indicates that lymphocytes are capable of synthesizing somatostatin, which may act as an autocrine or paracrine factor.

The modulation of immune functions by somatostatin

Somatostatin might modulate the immune response by a variety of mechanisms. These include modification of immunoglobulin secretion by plasma cells, suppression or enhancement of lymphocyte proliferation, cytotoxicity and changes in cytokine production, release of mediators by basophils, recruitment of eosinophils, and changes in macrophage function.

Somatostatin and immunoglobulin production

The murine IgA secreting plasmacytoma cell line MOPC-315 was found to bind fluorescent somatostatin as well as ^{125}I labelled somatostatin (25). This plasmacytoma cell line presented a high density of high affinity receptors (K_d = 1.6 nM). In contrast, another plasmacytoma cell line, J-558, expressed no somatostatin receptors. Somatostatin at a concentration of 10 nM inhibited the IgA synthesis by MOPC-315 significantly by 50%, while 1 pM of the peptide exerted a stimulatory action (+ 50%). Somatostatin inhibited the synthesis of IgA and IgM by lymphocytes prepared from Peyer's patches, mesenteric lymph nodes and the spleen (26). The synthesis of IgA was more inhibited (20-50%) than that of IgM (10-30%) (14,26).

All three immunoglobulin producing B lymphocyte isotypes in Peyer's patches bind somatostatin in a similar fashion (14). So, the inhibition of immunoglobulin production by somatostatin cannot be explained on the basis of binding to distinct subsets of B lymphocytes. Somatostatin inhibits all three classes of immunoglobulin production by unstimulated as well as pokeweed mitogen stimulated human peripheral blood lymphocytes. The highest percentage of inhibition was observed in the synthesis of IgG and IgA (27).

Summarizing, plasma cells express somatostatin receptors. Somatostatin inhibits immunoglobulin synthesis, and affects in particular the production of IgA. In view of this, somatostatin might exert a local regulatory influence on IgA synthesis by the gut.

The influence of somatostatin on lymphoid cell proliferation

Somatostatin analogues have direct antiproliferative effects on a variety of experimental human solid tumour cell lines (9). These inhibitory effects seem to be mediated via specific somatostatin receptors located on the tumour cells. Antiproliferative effects were reported on leukaemia cell lines and activated white blood cells as well. Plasma obtained from a patient with a somatostatinoma was shown to inhibit colony formation and tritiated thymidine incorporation by T lymphocytes (28). The somatostatin produced by this tumour was identified as somatostatin 14 and was suggested to have immunoregulatory properties. The influence of somatostatin on lymphocyte and lymphoid cell line proliferation was extensively studied, using cells originating from different species (Table 1). In most investigations (10 out of 16) somatostatin showed a concentration dependent modulation of the spontaneous and lectin induced proliferative response of lymphocytes and cell lines. At concentrations of 0.01-1 nM an inhibitory effect on the proliferation was observed. In three reports a biphasic proliferative response of lymphoid cells was observed. This response was antimitotic at low somatostatin concentrations, but became stimulatory at concentrations above 100 nM. At present the pathophysiological meaning of these biphasic responses is not known. Different somatostatin receptor subsets may be responsible for this phenomenon. We postulate that low numbers of high affinity receptors induce an inhibitory and high numbers of low affinity receptors mediate a stimulatory proliferative response. In other words, direct and indirect effects of somatostatin may have opposite results.

An interesting observation was the inhibition of lymphocyte proliferation after the in vivo administration of somatostatin and the powerful somatostatin analogue BIM-23014c (29). Mouse lymphocytes from the spleen and Peyer's patches demonstrated a reduction of [^{3}H]-thymidine incorporation by 50% and 65%, respectively. After exposure to somatostatin in vivo, and thereafter in vitro as well, a further reduction in the proliferative response was demonstrated by 85% and 93%, respectively. The higher numbers of somatostatin receptors on Peyer's patches might possibly have caused the difference in inhibition of proliferation between lymphocytes from Peyer's patches and lymphocytes from the spleen (29).

Table 1. The influence of somatostatin and analogues on the proliferation of lymphoid cells.

Cells/cell lines	Somatostatin concentration (in nM)		References
	Inhibition	Stimulation	
IgA secreting plasmacytoma MOPC-315	0.001 - 1	>100	25
ConA* stimulated murine lymphocytes (Peyer's patches, mesenteric lymph nodes, spleen)	>0.001		26,29-31
Human lamina propria lymphocytes	none	none	27,32
Human peripheral lymphocytes	0.01 - 1000		
PHA* stimulated human T lymphocytes	0.0001 - 1		33
Molt-4 lymphoblasts	0.0001 - 0.1	>100	
ConA* stimulated rat thymocytes	0.01 - 100		34
Spontaneous proliferation of mouse spleen lymphocytes	1 - 10	>100	35
Human thymocytes, guinea pig T lymphocytes, rat B lymphocytes	none	none	36
Rabbit spleen lymphocytes	none	100	37
ConA*/LPS* stimulated mouse lymph node lymphocytes	none	none	38
PHA*/LPS* stimulated rainbow trout lymphocytes	0.1 - 1000		39
Guinea pig spleen lymphocytes		<0.001 - 1000	40
Mercurous chloride stimulated peripheral T lymphocytes		1 - 1000	41,42

* ConA = concanavalin A; PHA = phytohaemagglutinin; LPS = lipopolysaccharide

The influence of somatostatin on the activation of isolated mononuclear cells, obtained from the human intestinal mucosa, were compared with peripheral blood lymphocytes (27). The amount of somatostatin required for a 50% inhibition of the phytohaemagglutinin induced expression of the interleukin 2 receptor was 100 to 1000 times lower in the intestinal mucosa population than in the peripheral blood lymphocytes. This somatostatin effect occurred early after mitogen stimulation, while lymphocytes tended to be partially refractory in the late phase of activation (27,40). The affinity of the somatostatin receptor on intestinal mucosa lymphocytes was approximately 500 times higher than on peripheral blood lymphocytes (K_d = 2.1 nM vs. K_d = 910 nM). This difference in affinity suggests the existence of two subtypes of somatostatin receptors, namely a low affinity receptor in resting lymphocytes and a high affinity receptor in activated lymphocytes. Inhibition of proliferation by somatostatin was less in peripheral blood lymphocytes than in lamina propria lymphocytes, an effect that might be explained by an interleukin independent alternative pathway (27).

The effects of somatostatin in many tissues have been related to the inhibition of cyclic adenosine monophosphate (cAMP) production (43-47). Somatostatin inhibits adenylate cyclase by a membrane receptor coupled to activation of an adenylate cyclase inhibiting G protein complex. Somatostatin in physiological doses does not influence the adenylate cyclase activity in lymphocytes and leukaemic cells but an inhibition in cAMP generation was observed in response to higher concentrations of somatostatin (48). These investigations suggest that the lymphocyte modulating effects of somatostatin cannot be ascribed to a decrease in intracellular cAMP level. However, in another study it was shown that the somatostatin analogue RC-102-2H decreases cAMP production in spleen lymphocytes (49).

Somatostatin receptors have been shown in many tissues to be linked to G proteins (50-52). Guanosine triphosphate (GTP) can reduce agonist binding to somatostatin receptors by uncoupling G proteins from the receptors, thereby converting the receptors to a low affinity state for agonists (50-52). GTP is able to inhibit the binding of [^{125}I-Tyr^3]-octreotide in the germinal centres of gut associated lymphoid tissues. Summarizing, most studies so far suggest that the somatostatin receptor in lymphoid tissue is coupled to a G protein, representing a true membrane somatostatin receptor.

The influence of somatostatin on cytotoxicity and cytokine production.

In vivo administration of somatostatin and the somatostatin analogue BIM-23014c affects the natural killer activity in vitro of lymphocytes from the mouse spleen and Peyer's patches (29). The inhibition of the killer activity was significantly higher in lymphocytes prepared from Peyer's patches than from the spleen. Krco et al. observed no influence of somatostatin on lymphocyte proliferation. However, at nanomolar concentrations an inhibitory effect was found on the mixed lymphocyte reaction (38). Somatostatin had no effect on the cytotoxic response against K-562 and CaCo-2 colon carcinoma target cells (53).

Apart from the influence of somatostatin on cellular cytotoxicity, the influence of somatostatin on the production of cytotoxic cytokines was also studied (54). The destruction of mitomycin C treated murine L-929 cells, induced by lymphotoxin and tumour necrosis factor, was enhanced by somatostatin 14 and especially by somatostatin 28. The observed cytotoxic effects in these experiments significantly increased over a narrow concentration range of somatostatin. The authors hypothesized that somatostatin promotes cell injury by inhibiting the repair mechanism of the affected cells.

Somatostatin and somatostatin analogues inhibit the secretion of hormones produced by normal endocrine cells as well as by neuroendocrine tumours. The release of both lymphotoxin and tumour necrosis factor by peripheral mononuclear cells was significantly decreased by 10-100 nM somatostatin (54). In another study it was shown that somatostatin and octreotide inhibit the secretion of interferon γ but not interferon α by mononuclear cells at concentrations higher than 1 μM (55). It seems unlikely that this effect occurs at physiological conditions because of the very high somatostatin concentration applied in these experiments, while somatostatin is also unable to decrease the interferon production induced by natural antigens (53). In contrast, somatostatin (10 nM) was shown to inhibit the mitogen induced production of interferon γ by schistosoma induced granulomas and splenic T lymphocytes by as much as 75% (56,57). This suggests a regulatory function of somatostatin in the granuloma response.

Summarizing, controversial reports have been published concerning a possible enhancement of cytotoxicity by somatostatin. Modification of cytokine production by somatostatin or somatostatin analogues may be a regulatory factor in the inflammatory response.

The influence of somatostatin on basophils and eosinophils

Somatostatin has been reported to suppress the antihuman myeloma IgE induced histamine and leukotriene release by human basophils, mouse bone marrow derived mast cells and rat leukaemic basophils at pico- to nanomolar concentrations (58,59). The rat leukaemic basophils released a somatostatin-like peptide, which suggests a role in the feedback regulation of basophil activity (60). In contrast to these results somatostatin 14 and other somatostatin analogues at much higher concentrations enhanced histamine release by mast cells (61). Such a histamine releasing effect of high concentrations (5 μM) of somatostatin 14 and somatostatin 28 was also described in white blood cells prepared from patients with allergic hyperreactivity (62). The pattern of histamine release suggests a biphasic effect with inhibition in the lower (nM) concentrations of somatostatin, as also described in the proliferation experiments mentioned above.

The hypereosinophilia in rat which can be induced by administration of cyclophosphamide or sephadex 48 hours before immunization with complete Freund's adjuvant, is significantly decreased by the somatostatin analogue BIM-23014c (63,64). Somatostatin has been shown to enhance the formation of human leucocyte migration inhibiting factor (65). This activity may account in part for its depressive effect on eosinophils.

Summarizing, somatostatin may be involved in hyperreactivity by regulation of mediator release by basophils and the recruitment of eosinophils. At low somatostatin concentrations an inhibitory effect was observed.

Somatostatin and macrophages

Circulating monocytes present low numbers of somatostatin receptors (12). Macrophages are able to destroy tumour cells. The tumoricidal capacity is

greatly enhanced after exposure to recombinant interferon. Peck showed that this effect was abolished in the presence of somatostatin (66). Concentrations of somatostatin needed to obtain this effect were above 1 nM with a maximal concentration at 10 μM. The author hypothesized that these high concentrations of somatostatin are needed because of protease activity in the culture medium and the rapid degradation of the peptide. In this study, the inhibitory effects of somatostatin could be reversed by substance P (66).

In another experiment, the chemotactic activity of monocytes was investigated (67). Chemotaxis was enhanced by octreotide but not by somatostatin 14. Patients with acromegaly, who were chronically treated with octreotide, showed an impaired chemotactic response, suggesting down regulation of somatostatin receptors (67). Octreotide also increases the phagocytic activity of monocytes from patients with cirrhosis of the liver (68).

Somatostatin at concentrations of 1-100 nM stimulated the lysis and inhibited the incorporation of IgG coated ^{51}Cr labelled sheep red blood cells by rat peritoneal macrophages (69). Higher concentrations resulted in an opposite effect, which again suggests a biphasic effect.

Summarizing, somatostatin and somatostatin analogues influence macrophage function. An interesting phenomenon is the difference between octreotide and somatostatin 14 affecting the chemotactic response, which suggests the presence of different receptor subtypes on monocytes.

Summary

Somatostatin and somatostatin receptors have been demonstrated in non-pathological and pathological lymphoid tissue and may play a regulatory, mostly inhibitory, role in the immune response. Somatostatin is produced by lymphocytes and monocytes which suggests an autocrine or paracrine regulatory role, but somatostatin may be released as well by nerve endings. It can therefore be hypothesized that apart from a role of somatostatin in local immunomodulation of cells belonging to the immune system, a second pathway exists in which this peptide exerts its effects via neuroendocrine modulation of the immune response. This might represent a direct regulatory relation between the nervous and the immune system.

References

1. Ottaway CA. Neuroimmunomodulation in the intestinal mucosa. Gastroenterol Clin North Am 1991;20:511-29.
2. Bienenstock J, Denburg J, Scicchitano R, Stead RH, Perdue M, Stanisz A. Role of neuropeptides, nerves and mast cells in intestinal immunity and physiology. Monogr Allergy 1988;24:134-43.
3. Bienenstock J, Croitoru K, Ernst PB, Stead RH, Stanisz A. Neuroendocrine regulation of mucosal immunity. Immunol Invest 1989;18:69-76.
4. O'Dorisio MS. Neuropeptide modulation of the immune response in gut associated lymphoid tissue. Int J Neurosci 1988;38:189-98.
5. Reichlin S. Somatostatin (first of two parts). N Eng J Med 1983;309:1495-1501.
6. Reichlin S. Somatostatin (second of two parts). N Eng J Med 1983;309:1556-63.
7. Kvols LK, Moertel CG, O'Connel MJ, Schutt AJ, Rubin J, Hahn RG. Treatment of malignant carcinoid syndrome. Evaluation of a long-acting somatostatin analogue. N Engl J Med 1986;315:663-6.
8. O'Dorisio TM, ed. Neuroendocrine disorders of the gastroenteropancreatic system: clinical applications of the somatostatin analogue SMS-201-995. Am J Med 1986;81:Suppl 6B:1-101.
9. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. Endocr Rev 1991;12:450-82.
10. Fedorak RN. Long acting somatostatin analogue: clinical potential for gastrointestinal disease. Can J Gastroenterol 1989;3:77-81.
11. Reubi JC, Kvols LK, Krenning EP, Lamberts SWJ. Distribution of somatostatin receptors in normal and tumor tissue. Metabolism 1990;39 Suppl 2:78-81.
12. Bhathena SJ, Louie J, Schechter GP, Redman RS, Wahl L, Recant L. Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 1981;30:127-31.
13. Hiruma K, Koike T, Nakamura H, Sumida T, Maeda T, Tomioka H, Yoshida S, Fujita T. Somatostatin receptors on human lymphocytes and leukaemia cells. Immunology 1990;71:480-5.
14. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987;1:173-84.
15. Sreedharan SP, Kodama KT, Peterson KE, Goetzl EJ. Distinct subsets of somatostatin receptors on cultured human lymphocytes. J Biol Chem 1989;264:949-52.
16. Nakamura H, Koike T, Hiruma K, Sato T, Tomioka H, Yoshida S. Identification of lymphoid cell lines bearing receptors for somatostatin. Immunology 1987;62:655-8.
17. Brownstein M, Arimura A, Sato H, Schally AV, Kizer JS. The regional distribution of somatostatin in the rat brain. Endocrinology 1975;96:1456-61.
18. Kobayashi RM, Brown M, Vale W. Regional distribution of neurotensin and somatostatin in rat brain. Brain Res 1977;126:584-8.
19. Payan DG, Goetzl EJ. Neuropeptide regulation of immediate and delayed hypersensitivity. Int J Neurosci 1988;38:211-21.

20. Felten DL, Felten SY, Carlson SL, Olschowka JA, Livnat S. Noradrenergic and peptidergic innervation of lymphoid tissue. J Immunol 1985;135 Suppl:755s-65s.
21. Popper P, Mantyh CR, Vigna SR, Maggio JE, Mantyh PW. The localization of sensory nerve fibers and receptor binding sites for sensory neuropeptides in canine mesenteric lymph nodes. Peptides 1988;9:257-67.
22. Reubi JC, Horisberger U, Waser B, Gebbers JO, Laissue J. Preferential location of somatostatin receptors in germinal centers of human gut lymphoid tissue. Gastroenterology 1992;103:1207-14.
23. Aguila MC, Dees WL, Haensly WE, McCann SM. Evidence that somatostatin is localized and synthesized in lymphoid organs. Proc Natl Acad Sci U S A 1991;88:11485-9.
24. Fuller PJ, Verity K. Somatostatin gene expression in the thymus gland. J Immunol 1989;143:1015-7.
25. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. J Immunol 1988;141:937-941.
26. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
27. Fais S, Annibale B, Boirivant M, Santoro A, Pallone F, Delle Fave G. Effects of somatostatin on human intestinal lamina propria lymphocytes. J Neuroimmunol 1991;31:211-9.
28. Vehmeyer K, Schuff-Werner P, Nagel GA. Suppressed colony formation of peripheral blood lymphocytes in a patient with somatostatinoma. Clin Immunol Immunopathol 1986;41:290-4.
29. Agro A, Padol I, Stanisz AM. Immunomodulatory activities of the somatostatin analogue BIM-23014c: effects on murine lymphocyte proliferation and natural killer activity. Regul Pept 1991;32:129-39.
30. Scicchitano R, Bienenstock J, Stanisz AM. The differential effect with time of neuropeptides on the proliferative responses of murine Peyer's patch and splenic lymphocytes. Brain Behav Immun 1987;1:231-7.
31. Stanisz AM, Scicchitano R, Payan DG, Bienenstock J. In vitro studies of immunoregulation by substance P and somatostatin. Ann N Y Acad Sci 1987;496:217-25.
32. Malec P, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmacology 1989;17:45-9.
33. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.
34. Mascardo RN, Barton RW, Sherline P. Somatostatin has an antiproliferative effect on concanavalin A-activated rat thymocytes. Clin Immunol Immunopathol 1984;33:131-8.
35. Pawlikowski M, Stepien H, Kunert-Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985;129:52-5.

36. Söder O, Hellström PM. Neuropeptide regulation of human thymocyte, guinea pig T lymphocyte and rat B lymphocyte mitogenesis. Int Arch Appl Allergy Immunol 1987;84:205-11.
37. Peuriere S, Susini C, Alvinerie M, Vaysse N, Escoula L. Interactions between neuropeptides and dexamethasone on the mitogenic response of rabbit spleen lymphocytes. Peptides 1991;12:645-51.
38. Krco CJ, Gores A, Go VL. Gastrointestinal regulatory peptides modulate in vitro immune reactions of mouse lymphoid cells. Clin Immunol Immunopathol 1986;39:308-18.
39. Ndoye A, Troutaud D, Rougier F, Deschaux P. In vitro effects of substance P and somatostatin on lymphoproliferation in rainbow trout (Salmo gairdneri). Ann N Y Acad Sci 1992;650:160-4.
40. Johansson O, Sandberg G. Effect of theeuropeptides beta-MSH, neurotensin, NPY, PHI, somatostatin and substance P on proliferation of lymphocytes in vitro. Acta Physiol Scand 1989;137:107-11.
41. Nordlind K, Mutt V. Influence of beta-endorphin, somatostatin, substance P, and vasoactive intestinal peptide on the proliferative response of human peripheral blood T lymphocytes to mercuric chloride. Int Arch Allergy Immunol 1986;80;326-8.
42. Nordlind K, Mutt V. Modulating effect of beta-endorphin, somatostatin, substance P and vasoactive intestinal peptide on the proliferative response of peripheral blood T lymphocytes of nickel-allergic patients to nickel sulfate. Int Arch Allergy Appl Immunol 1986;81:368-70.
43. Borgeat P, Labrie F, Drouin J, Belanger A, Immer H, Sestanj K, Nelson V, Gotz M, Schally A, Coy DH, Coy EJ. Inhibition of adenosine 3',5'-monophosphate accumulation in anterior pituitary gland in vitro by growth hormone release inhibiting hormone. Biochem Biophys Res Commun 1974;56:1052-9.
44. Kaneko T, Oka H, Munemure M, Suzuki S, Yasuda H. Stimulation of guanosine 3',5'-cyclic monophosphate accumulation in rat anterior pituitary gland in vitro by synthetic somatostatin. Biochem Biophys Res Commun 1974;61:53-7.
45. Efendic S, Grill V, Luft R. Inhibition by somatostatin of glucose induced 3',5'-monophosphate (cyclic AMP) accumulation and insulin release in isolated pancreatic islets of the rat. FEBS Lett 1975;55:131-3.
46. Gespach C, Dupont C, Bataille D, Rosselin G. Selective inhibition by somatostatin of cyclic AMP production in rat gastric glands: demonstration of a direct effect on the parietal cell function. FEBS Lett 1980;114:247-52.
47. Chneiweiss H, Glowinski J, Premont J. Modulation by monoamine of somatostatin-sensitive adenylate cyclase on neuronal and glial cells from the mouse brain in primary cultures. J Neurochem 1985;44:1825-31.
48. Perracchi M, Bamonti-Catena, Bareggi B. Effect of high doses of somatostatin on adenylate cyclase activity in peripheral mononuclear leucocytes from normal subjects and from acute leukaemia patients. Experientia 1988;44:613-5.
49. Pawlikowski M, Stepien H, Kunert-Radek J, Zelazowski P, Schally AV. Immunomodulatory action of somatostatin. Ann N Y Acad Sci 1987;496:233-9.
50. Enjalbert A, Rasolon-Janahary R, Moyse E, Kordon C, Epelbaum J. Guanine nucleotide sensitivity of [^{125}I-Tyr^{11}]-somatostatin binding in rat adenohypophysis and cerebral cortex. Endocrinology 1983;113:822-4.

51. Raynor K, Reisine T. Analogs of somatostatin selectively label distinct subtypes of somatostatin receptors in rat brain. J Pharmacol Exp Ther 1989;251:510-7.
52. Marie JC, Cotroneo P, de Chasseval R, Rosselin G. Solubilization of somatostatin receptors in hamster pancreatic beta cells. Characterization as a glycoprotein interacting with a GTP-binding protein. Eur J Biochem 1989;186:181-8.
53. van Tol AF, Verspaget HW, Lamers CB. Neuropeptide regulation of cell mediated cytotoxicity against human tumor cells. Neuropeptides 1990;16:25-32.
54. Yousefi S, Vaziri N, Carandang G, Le W, Yamamoto R, Granger G, Ocariz J, Cesario T. The paradoxical effects of somatostatin on the bioactivity and production of cytotoxins derived from human peripheral blood mononuclear cells. Br J Cancer 1991;64:243-6.
55. Yousefi S, Ghazinouri A, Vaziri N, Tilles J, Carandang G, Cesario T. The effect of somatostatin on the production of human interferons by mononuclear cells. Proc Soc Exp Biol Med 1990;194:114-8.
56. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. [Review] Clin Immunol Immunopathol 1992;64:17-22.
57. Blum AM, Metwali A, Mathew RC, Cook G, Elliott D, Weinstock JV. Granuloma T lymphocytes in murine schistosomiasis mansoni have somatostatin receptors and respond to somatostatin with decreased IFN-gamma secretion. J Immunol 1992;149:3621-6.
58. Goetzl EJ, Payan DG. Inhibition by somatostatin of the release of mediators from human basophils and rat leukemic basophils. J Immunol 1984;133:3255-9.
59. Renold F, Chernov T, Lee J, Payan DG, Furuichi K, Goetzl EJ. Somatostatin (SOM 14) modulation of mediator release by mouse bone marrow derived mast cells (BMMC), rat serosal mast cells (SMC), and rat basophilic leukaemia cells (RBL-2H3). Fed Proc 1985;44:1917.
60. Chernov-Rogan T, Lee J, Payan DG, Goetzl EJ. Endogenous somatostatin-like peptides of rat basophilic leukaemia cells. Clin Res 1985;33:515.
61. Theoharides TC, Douglas WW. Mast cell histamine secretion in response to somatostatin analogues: structural considerations. Eur J Pharmacol 1981;73:131-6.
62. Diel F, Bethge N, Opree W. Histamine secretion in leukocyte incubates of patients with allergic hyperreactivity induced by somatostatin-14 and somatostatin-28. Agents Actions 1983;13:216-8.
63. Etienne A, Soulard C, Thonier F, Braquet P. Modulation by drugs of eosinophil recruitment induced by immune challenge in the rat. Possible roles of interleukin 5 and platelet-activating factor. Int Arch Allergy Appl Immunol 1989;88:216-21.
64. Etienne A, Soulard C, Thonier F, Braquet P. Modulation of eosinophil recruitment in the rat by the platelet-activating factor (PAF) antagonist, BN-52021, the somatostatin analog BIM-23014, and by cyclosporin A. Prostaglandins 1989;37:345-57.
65. Pawlikowski M, Zelazowski P, Stepien H, Schally AV. Somatostatin and its analog enhance the formation of human leukocyte migration inhibiting factor: further evidence for immunomodulatory action of somatostatin. Peptides 1987;8:951-2.
66. Peck R. Neuropeptides modulating macrophage function. Ann N Y Acad Sci 1987;496:264-70.

67. Sacerdote P, Bianchi M, Panerai AE. Human monocyte chemotactic activity of calcitonin and somatostatin related peptides: modulation by chronic peptide treatment. J Clin Endocrinol Metab 1990;70:141-8.
68. Jenkins SA, Baxter JN, Day DW, Al-Sumidaie AM, Leinster SJ, Shields R. The effects of somatostatin and SMS-201-995 on experimentally induced pancreatitis and endotoxaemia in rat and on monocyte activity in patients with cirrhosis and portal hypertension. Klin Wochenschr 1986;64 Suppl 7:100-6.
69. Foris G, Gyimesi E, Komaromi I. The mechanism of antibody-dependent cellular cytotoxicity stimulation by somatostatin in rat peritoneal macrophages. Cell Immunol 1985;90:217-25.

CHAPTER 3

SOMATOSTATIN RECEPTORS IN MALIGNANT LYMPHOMAS

3.1 Somatostatin receptor autoradiography in malignant lymphomas

3.2 Somatostatin receptor scintigraphy in malignant lymphomas

3.3 Somatostatin receptors in malignant lymphomas

CHAPTER 3.1

SOMATOSTATIN RECEPTOR AUTORADIOGRAPHY IN MALIGNANT LYMPHOMAS

Abstract

A wide variety of primary and metastatic human neoplasms express somatostatin receptors. We evaluated the somatostatin receptor status of malignant lymphomas that had been surgically removed from 31 patients. Somatostatin receptors were visualized by in vitro somatostatin receptor autoradiography with the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide as radioligand. Of eleven low grade malignancy B cell non-Hodgkin's lymphomas, ten were somatostatin receptor positive, with a high receptor density restricted to the neoplastic follicles. All of the eight intermediate grade non-Hodgkin's lymphomas were somatostatin receptor positive. Seven out of ten non Hodgkin's lymphomas of high grade malignancy were somatostatin receptor positive, often with a high density of receptors. One T cell lymphoma and one Hodgkin's lymphoma were also positive. Somatostatin receptors were of high affinity (K_d = 1.2 nM) and specific for bioactive somatostatin analogues.

Introduction

Somatostatin receptors have been observed not only in normal somatostatin target tissues, but also in several types of human cancer (1). The receptors occur in most neuroendocrine neoplasms (2,3), but also in tumours of the central nervous system (4,5), breast (6,7) and lung (8). The presence of somatostatin receptors may be, for certain tumour types, a pathobiochemical marker of predictive value for the efficacy of somatostatin analogue therapy (3,9), while it may also be of prognostic significance (10). Moreover, these somatostatin receptors can be visualized in vivo with gamma camera scanning techniques, enabling the precise localization of primary and metastatic tumour sites (11-13).

It has been known for several years that the lymphopoietic system may be a target for somatostatin. Indeed, somatostatin affects various lymphocytic functions in non-neoplastic and neoplastic murine or human lymphoid cells in vitro (14,15). For instance, somatostatin has been shown to inhibit lymphocyte proliferation (16-20), forskolin stimulated adenylate cyclase (21,22), as well as IgA synthesis in lymphocytes (20). In addition, somatostatin antagonizes actions of vasoactive intestinal peptide and substance P in lymphoid tissues (14). These effects may be mediated via specific receptors, since somatostatin receptors

have been found in several types of non-neoplastic lymphocytes in mice (23), in various lymphoid cell lines, such as the Jurkat line of human leukaemic T cells, in IgE producing U-266 myeloma cells (24) and in the MT-2, Isk and Molt-4F cell lines (25). Peripheral resting human lymphocytes also possess somatostatin receptors, albeit of lower affinity (26). The biochemical processes mediated by somatostatin through its receptors are likely to be the molecular basis for a wide range of somatostatin actions affecting the immune system (14,15,27,28).

The aim of the present study was to investigate, using in vitro receptor autoradiography, whether human malignant lymphomas, mostly non-Hodgkin's lymphomas, express somatostatin receptors.

Materials and methods

Patients

Thirty-four surgical biopsies from 31 patients with lymphoma were obtained from the Institutes of Pathology of Berne and Lucerne, Switzerland, and from the Department of Internal Medicine, Erasmus University Hospital Rotterdam, the Netherlands. One part of the material was fixed in neutral buffered formalin and used for histopathological and immunohistochemical study. Another part was frozen and used for somatostatin receptor autoradiography. Tumours were classified in accordance with either the International Working Formulation system (29) or the Kiel classification (30). Low grade lymphoma tissue was obtained from 12 patients (13 samples), lymphomas of intermediate grade from 8 patients (9 samples), and high grade lymphomas from 10 patients (11 samples). One sample from a patient with Hodgkin's disease was included. Non-neoplastic lymphoid tissues, including lymph nodes with minor histopathological changes and lymph nodes bearing non-lymphomatous metastatic tumours, were also examined.

Somatostatin receptor autoradiography

Receptor autoradiography was done on cryostat sections (10 μm and 20 μm) of the tumour samples, using [^{125}I-Tyr^{3}]-octreotide as radioligand, as described previously (2,3). Briefly, the sections were mounted on pre-cleaned microscope slides and stored at -20°C for at least 3 days to improve adhesion of tissue to the slide. Sections were then incubated for 2 hours at room temperature in the

presence of the iodinated ligand (0.15-0.30 x 10^6 dpm/ml, about 80-160 pM). The incubation solution was 170 mM Tris-HCl buffer, pH 7.4, containing 1% bovine serum albumin (BSA), bacitracin (40 μg/ml), and $MgCl_2$ (5 mM) to inhibit endogenous proteases. Non-specific binding was determined by adding 1 μM solution of the unlabelled peptide [Tyr^3]-octreotide. Incubated sections were washed twice for 5 minutes in cold incubation buffer containing 0.25% BSA, then in buffer alone, and dried quickly. Compared to previous procedures of washing in distilled water (4,5,7), the present method of washing in buffer resulted in a much better histological preservation of the lymphoid tissues. Finally, the sections were apposed to ^3H labelled ultrafilms (Cambridge Research, Nussdorf, Germany) and exposed for one week in X-ray cassettes. In selected cases displacement experiments were done in successive sections of a tumour by increasing the concentration of various biologically active or inactive peptides (3). In addition, saturation experiments using increasing concentrations of [^{125}I-Tyr^3]-octreotide were performed on tumour tissue sections (3). The autoradiograms were quantified using a computer assisted image processing system (3). Tissue standards for iodinated compounds (Amersham, Aylesbury, United Kingdom) were used for this purpose. A tumour was defined as somatostatin receptor positive when the optical density measured over a tumour area in the 'total binding' section was at least twice the optical density of the 'non-specific' binding section.

Immunohistochemistry

Paraffin sections, 5-7 μm thick, were mounted on glass slides coated with polyvinyl-acetate glue, de-paraffinized, hydrated and, if required, treated with 0.1% trypsin for 10 minutes at 37°C. Sections were then washed and incubated for 18-24 hours at room temperature with specific antibodies directed against CD antigens of lymphocytes: (a) lymphoid cells: CD45 (antibody LC); (b) B lymphocytes: CD20 and 45R (antibodies L26, 4KB5, KiN3, MB1); (c) T lymphocytes: CD4, CD5 and 45RO (antibodies T1, T4, UCHL1); (d) CD30 (antibody: Ber-H2); and (e) immunoglobulin light chains (antibodies kappa, lambda). Incubations without specific antibodies served as negative controls. The avidin-biotin-peroxidase complex technique was used (31).

Results

Table 1 describes the results of the somatostatin receptor determination in the three different groups of non-Hodgkin's lymphoma. In lymphomas of low grade malignancy 11 out of 12 cases (92%) were somatostatin receptor positive. All lymphomas of intermediate grade malignancy were positive. Among ten lymphomas of high grade malignancy, seven were positive for somatostatin receptors (70%). In addition, a tissue sample of Hodgkin's disease with nodular sclerosis was somatostatin receptor positive. Overall, a low to moderate density of receptors was observed, but several samples, particularly lymphomas of high grade malignancy, expressed a high somatostatin receptor density.

Figure 1 shows a low grade follicular lymphoma, in which the follicles were preferentially and heavily labelled, whereas the surrounding cells and interfollicular cells had a low receptor density, or no demonstrable somatostatin receptors. As seen in Figure 2, the follicles displaying somatostatin receptors in this tumour sample contained mainly B lymphoid cells, with monotypic cytoplasmic immunoglobulin (not shown). Figure 3 shows an example of a high grade lymphoma with diffusely distributed somatostatin receptors. The somatostatin receptors in lymphoma tissues had high affinity for and were specific to somatostatin analogues, since unrelated peptides such as luteinizing hormone-releasing hormone did not influence the radioligand binding (Figure 4). Scatchard analysis of data obtained in saturation experiments using [^{125}I-Tyr^{3}]-octreotide gave a dissociation constant (K_d) of 1.2 nM in a B cell non-Hodgkin's malignant lymphoma. For comparison and control, non-lymphomatous lymphoid tissues, such as lymph nodes without neoplastic changes, lymph nodes with foci of non-lymphomatous metastatic tumours, or gastrointestinal lymphoid tissues, were also investigated. Whereas small lymphocytes and primary lymphatic follicles were usually somatostatin receptor negative, the central part of larger, non-neoplastic germinal centres was somatostatin receptor positive. This suggests that lymphoid cells expressing somatostatin receptors are frequently in a proliferative and/or stimulated stage.

Table 1. Somatostatin receptor autoradiography in non-Hodgkin's lymphoma of different malignancy grades.

Patient	Sex*	Age	Classification†	Therapy	Site of lymphoma	Autoradiography‡	
Low grade malignancy							
1	M	38	Small lymphocytic		Leg	+	Diffuse
2	F	52	Follicular, predominantly small cleaved cell		Thorax	+++	Follicular
3	M	57	Follicular, predominantly small cleaved cell		Neck	++	Follicular
4	M	54	Follicular, mixed small cleaved / large cell	Chemotherapy	Groin, axilla	+	Follicular
5	F	43	Follicular, predominantly small cleaved cell		Neck	+	Follicular
6	M	75	Follicular, predominantly small cleaved cell		Neck	+	Follicular
7	M	67	Follicular, predominantly small cleaved cell Follicular, mixed small cleaved / large cell		Neck	+	Follicular
8	F	40	Follicular, predominantly small cleaved cell		Groin	+	
9	M	60	Follicular, mixed small cleaved / large cell Diffuse, mixed small cleaved / large cell		Supraclavicular	+	
10	F	79	Follicular, mixed small cleaved / large cell Diffuse, mixed small cleaved / large cell		Neck	+	Diffuse
11	M	78	Follicular, mixed small cleaved / large cell Diffuse, mixed small cleaved / large cell		Upper arm	-	
12	M	77	T cell, angioimmunoblastic§		Supraclavicular	+	

Continued...

Table 1. (cont.)

Patient	Sex*	Age	Classification†	Therapy	Site of lymphoma	Autoradiography‡	
Intermediate grade malignancy							
13	F	45	Diffuse, mixed small cleaved / large cell Diffuse, large cell: cleaved or noncleaved	Chemotherapy	Lymph node	+++	Diffuse
14	M	63	Diffuse, large cell: cleaved or noncleaved		Supraclavicular	+++	Diffuse
15	F	72	Diffuse, large cell: cleaved or noncleaved	Chemotherapy	Axilla	++	Diffuse
16	M	29	Diffuse, mixed small cleaved / large cell		Liver hilus	+	Diffuse
17	F	62	Diffuse, mixed small cleaved / large cell	Radiotherapy	Axilla	+	Diffuse
18	F	90	Diffuse, mixed small cleaved / large cell		Tonsil, palatinum	+	Diffuse
19	M	63	Follicular, predominantly large cell Diffuse, large cell: cleaved or noncleaved		Abdomen	+	
20	M	62	Diffuse, mixed small cleaved / large cell		Neck	+	Diffuse

Continued...

Table 1. (cont.)

Patient	Sex*	Age	Classification†	Therapy	Site of lymphoma	Autoradiography‡	
High grade malignancy							
21	F	30	Diffuse, large cell: cleaved or noncleaved Diffuse, large cell immunoblastic		Small intestine	+++	Diffuse
22	M	56	B cell, centroblastic§	Chemotherapy Radiotherapy	Kidney	++	
23	M	13	Small non-cleaved cell: Burkitt's		Supraclavicular	++	
24	F	77	Diffuse, mixed small cleaved / large cell Diffuse, large cell immunoblastic		Neck	++	
25	M	81	B cell, centroblastic§		Neck	+	
26	F	37	Diffuse, large cell immunoblastic	Chemotherapy	Neck	+	
27	F	76	B cell, centroblastic§		Skin	+	Diffuse
28	F	62	B cell, centroblastic§		Axilla	-	
29	F	5	Small non-cleaved cell: Burkitt's		Small intestine	-	
30	M	3	Small non-cleaved cell: Burkitt's		Ileum	-	

* M = male; F = female

† International Working Formulation system

‡ +++ = high density of somatostatin receptors; ++ = moderate density of somatostatin receptors; + = low density of somatostatin receptors; - = no somatostatin receptors detectable

§ Kiel classification

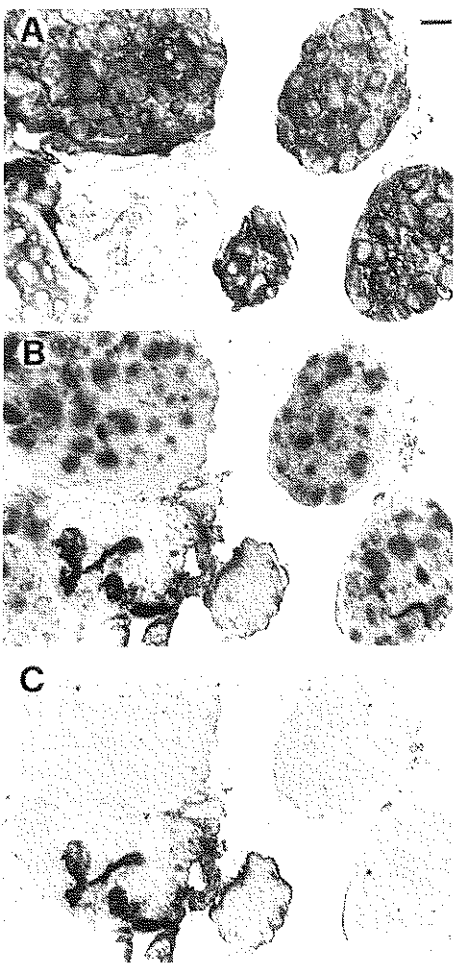

Figure 1. Visualization of somatostatin receptors in a low grade B cell lymphoma (patient 2, see Table 1). (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide in the presence of 1 μM [Tyr^3]-octreotide. Bar represents 1 mm.

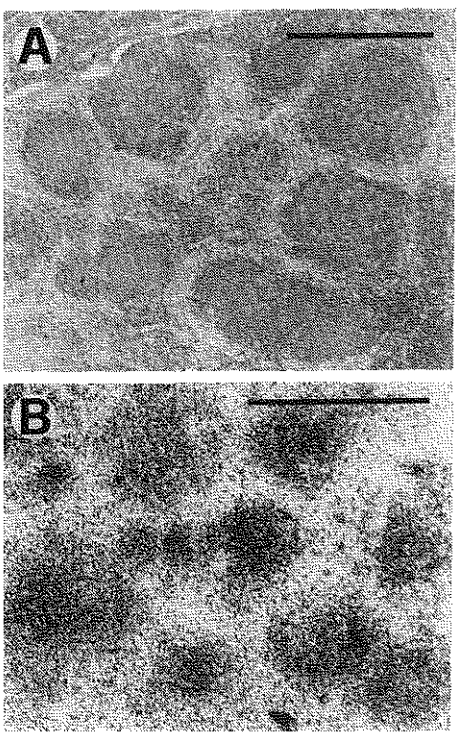

Figure 2. Low grade B cell lymphoma (patient 2, see Table 1). (A) Immunohistochemical staining for B cell type lymphoid cells. (B) Somatostatin distribution. Bars represent 1 mm.

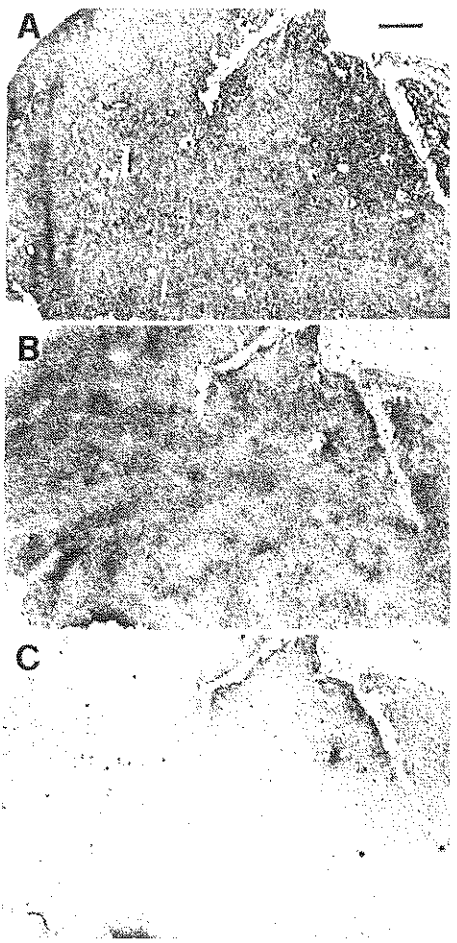

Figure 3. Somatostatin receptors in a high grade non-Hodgkin's lymphoma (patient 22, see Table 1). (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide in the presence of 1 μM [Tyr^3]-octreotide. Bar represents 1 mm.

Discussion

This study describes the presence of somatostatin receptors in most of the non-Hodgkin's human lymphoma tissues examined in vitro by receptor autoradiographic techniques. These receptors have high affinity for and are pharmacologically specific to somatostatin. They have been found in lymphomas of low, intermediate and high grade malignancy.

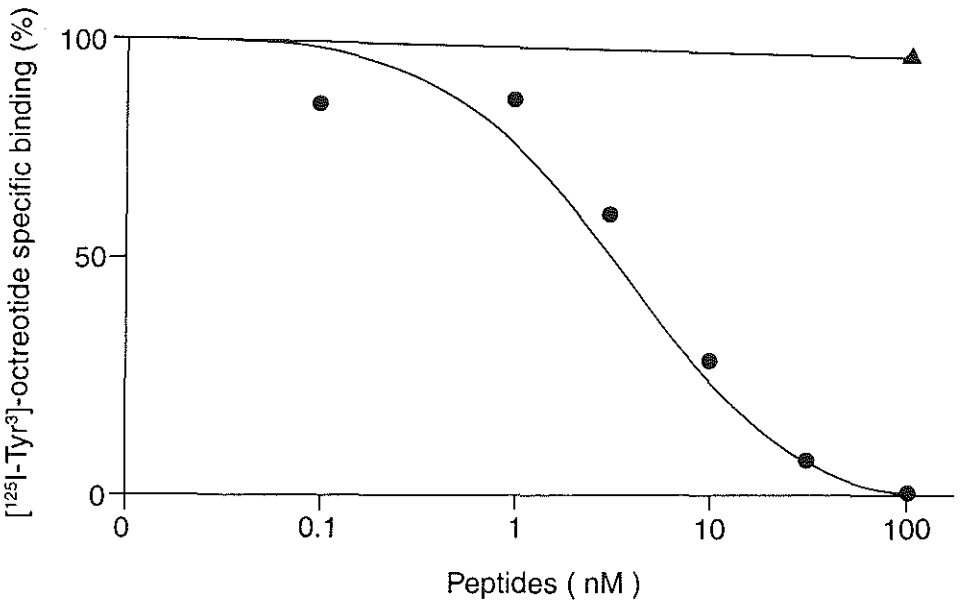

Figure 4. Somatostatin receptors in non-Hodgkin's lymphoma of intermediate grade (patient 13, see Table 1). Displacement curve of [^{125}I-Tyr^3]-octreotide in tumour tissue sections incubated with radioligand (30,000 counts per minute/100 µl) and increasing concentrations of unlabelled [Tyr^3]-octreotide (●) or 100 nM luteinizing hormone-releasing hormone (▲).

Various types of cells may express somatostatin receptors, depending on the lymphoma type and grade of malignancy. For instance, in low grade B cell lymphomas with a follicular pattern, the somatostatin receptor distribution was clearly non-homogeneous. The follicular structures consisted mainly of B cells rich in somatostatin receptors, whereas the interfollicular areas with many cells of the T phenotype displayed fewer somatostatin receptors. However, one high grade T cell lymphoma in the present series was also clearly positive for somatostatin receptors. Thus, neoplastic B and T lymphocytes have the potential of expressing somatostatin receptors under certain conditions. In most high grade lymphomas of the B cell type, the relatively homogeneous distribution of the somatostatin receptors reflected the diffuse histopathological pattern of these tumours.

The relatively frequent occurrence in high grade lymphomas of a moderate to high density of somatostatin receptors contrasts partially with previous somatostatin receptor studies of other neoplasms, such as glial tumours (5,32), or carcinoids (3). The expression of somatostatin receptors in these tumours was inversely related to their differentiation grade; most neuroendocrine tumours that displayed somatostatin receptors were usually well differentiated.

Few lymphomas examined in vitro lacked somatostatin receptors. Since these were mainly tumours with a high malignancy grade, the absence of somatostatin receptors may relate to analogous observations made in other tumour types (3,5,9). However, it may be due to other causes: (a) accidental causes, inadequate handling of materials (e.g. thawing of tissues before autoradiography), or (b) previous chemo- or radiotherapy. However, none of the somatostatin receptor negative tumour samples were acquired from patients treated by irradiation or given cytotoxic drugs. Moreover, the tumours of six patients given either chemo- or radiotherapy, or both, displayed somatostatin receptors.

The significance of somatostatin receptors in human malignant lymphoma is unknown. Some links between somatostatin, somatostatin receptors and characteristics of neoplastic lymphoid cells have been demonstrated in animals and humans. Studies in murine and human lymphoid cells and tissues have described various cellular actions of somatostatin, such as inhibition of cell proliferation (16-18,20), of IgA synthesis (20) and of forskolin stimulated adenylate cyclase production (21,22), and antagonism of the actions of vasoactive intestinal peptide and substance P in lymphatic tissues (14). It can therefore be expected that lymphocytes treated with somatostatin will undergo changes in their immunomodulatory characteristics (14,15,27). Since these actions are mediated by specific somatostatin receptors on the cell membrane (22,24,27), it can be speculated that similar actions of somatostatin might occur in human malignant lymphomas via the somatostatin receptors described. The potential therapeutic relevance of direct effects of somatostatin on malignant lymphoma could be evaluated using somatostatin analogues such as octreotide. In addition, indirect effects, i.e. more general consequences of somatostatin analogues on the normal immune system, should be considered (27,28).

The present study shows that somatostatin receptors may represent a general pathobiochemical marker for malignant lymphomas in vitro. Somatostatin

receptors may have useful diagnostic functions in vivo. We have already shown that several somatostatin receptor containing human tumours can be visualized in vivo by means of gamma camera scintigraphy after injection of ^{123}I- or ^{111}In labelled octreotide analogues (11,12,33). The in vivo labelling may be diagnostically helpful for a more sensitive staging of disease, and might be instrumental in improving the choice of therapy for various stages of disease in patients with malignant lymphomas.

References

1. Reubi JC, Kvols LK, Krenning EP, Lamberts SWJ. Distribution of somatostatin receptors in normal and tumor tissue. Metabolism 1990;39 Suppl 2:78-81.
2. Reubi JC, Heitz PU, Landolt AM. Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses. J Clin Endocrinol Metab 1987;65:65-73.
3. Reubi JC, Kvols LK, Waser B, Nagorney DM, Heitz PU, Charboneau JW, Reading CC, Moertel DC. Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. Cancer Res 1990;5O:5969-77.
4. Reubi JC, Maurer R, Klijn JGM, Stefanko SZ, Foekens JA, Blaauw G, Blankenstein MA, Lamberts SWJ. High incidence of somatostatin receptors in human meningiomas: biochemical characterization. J Clin Endocrinol Metab 1986;63:433-8.
5. Reubi JC, Lang W, Maurer R, Koper JW, Lamberts SWJ. Distribution and biochemical characterization of somatostatin receptors in tumors of the human central nervous system. Cancer Res 1987;47:5758-64.
6. Fekete M, Wittliff JL, Schally AV. Characteristics and distribution of receptors for [D-Trp^6]-luteinizing hormone-releasing hormone, somatostatin, epidermal growth factor, and sex steroids in 500 biopsy samples of human breast cancer. J Clin Lab Anal 1989;3:137-47.
7. Reubi JC, Waser B, Foekens JA, Klijn JGM, Lamberts SWJ, Laissue JA. Somatostatin receptor incidence and distribution in breast cancer using receptor autoradiography: relationship to EGF-receptors. Int J Cancer 1990;46:416-20.
8. Reubi JC, Waser B, Sheppard M, Macaulay V. Somatostatin receptors are present in small-cell but not in non-small-cell primary lung carcinomas: relationship to EGF receptors. Int J Cancer 1990;45:269-74.
9. Reubi JC, Landolt AM. The growth hormone responses to octreotide in acromegaly correlate with adenoma somatostatin receptor status. J Clin Endocrinol Metab 1989;68:844-50.
10. Foekens JA, Portengen H, van Putten WLJ, Trapman AMAC, Reubi JC, Alexieva-Figush J, Klijn JGM. Prognostic value of receptors for insulin-like growth factor 1, somatostatin, and epidermal growth factor in human breast cancer. Cancer Res 1989;49:7002-9.
11. Krenning EP, Bakker WH, Breeman WAP, Koper JW, Kooij PPM, Ausema L, Lameris JS, Reubi JC, Lamberts SWJ. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989;1:242-4.

12. Lamberts SWJ, Hofland LJ, van Koetsveld PM, Reubi JC, Bruining HA, Bakker WH, Krenning EP. Parallel in vivo and in vitro detection of functional somatostatin receptor in human endocrine pancreatic tumors: consequences with regard to diagnosis, localization and therapy. J Clin Endocrinol Metab 1990;71:566-74.
13. Bakker WH, Krenning EP, Breeman WAP, Koper JW, Kooij PPM, Reubi JC, Klijn JG, Visser TJ, Docter R, Lamberts SWJ. Receptor scintigraphy with a radioiodinated somatostatin analogue: radiolabeling, purification, biological activity, and in vivo application in animals. J Nucl Med 1990;31:1501-9.
14. O'Dorisio MS. Neuropeptides and gastrointestinal immunity. Am J Med 1986;81:74-82.
15. Pawlikowski M, Stepien H, Kunert-Radek J, Zelazowski P, Schally AV. Immunomodulatory action of somatostatin. Ann N Y Acad Sci 1987;496:233-9.
16. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.
17. Mascardo RN, Barton RW, Sherline P. Somatostatin has an antiproliferative effect on concanavalin A-activated rat thymocytes. Clin Immunol Immunopathol 1984;33:131-8.
18. Pawlikowski M, Stepien H, Kunert-Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985;129:52-5.
19. Krco CJ, Gores A, Go VL. Gastrointestinal regulatory peptides modulate mouse lymphocyte functions under serum-free conditions in vitro. Immunol Invest 1986;15:103-11.
20. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
21. Aktories K, Schultz G, Jakobs K. Somatostatin-induced stimulation of a high-affinity GTPase in membranes of S49 lymphoma cyc- and H21A variants. Mol Pharmacol 1983;24:183-8.
22. Mayor F Jr., Benovic JL, Caron MG, Lefkowitz RJ. Somatostatin induces translocation of the beta-adrenergic receptor kinase and desensitizes somatostatin receptors in S49 lymphoma cells. J Biol Chem 1987;262:6468-71.
23. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987;1:173-84.
24. Sreedharan SP, Kodama KT, Peterson KE, Goetzl EJ. Distinct subsets of somatostatin receptors on cultured human lymphocytes. J Biol Chem 1989;264:949-52.
25. Nakamura H, Koike T, Hiruma K, Sato T, Tomioka H, Yoshida S. Identification of lymphoid cell lines bearing receptors for somatostatin. Immunology 1987;62:655-8.
26. Bhathena SJ, Louie J, Schechter GP, Redman RS, Wahl L, Recant L. Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 1981;30:127-31.
27. Malec PH, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmacology 1989;17:45-9.
28. Ritts RE, Kvols LK, Strehlo B, Jacobsen D, Patel S. Immunologic studies of patients with malignant neuroendocrine carcinomas and responses to somatostatin analogue (SMS 201-995, Sandostatin™) [abstract]. Proc Am Ass Cancer Res 1989;30 Abstr 1119:281.

29. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer 1982;49:2112-35.
30. Stansfeld AG, Diebold J, Noel H, Kapancy Y, Rilke F, Kelenyi G, Sundstrom C, Lennert K, van Unnik JAM, Mioduszewska O, Wright DH. Updated Kiel classification for lymphomas [letter]. Lancet 1988;1:1292-3.
31. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and the unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981;29:577-80.
32. Reubi JC, Horisberger U, Lang W, Koper JW, Braakman R, Lamberts SWJ. Coincidence of EGF receptors and somatostatin receptors in meningiomas but inverse, differentiation-dependent relationship in glial tumors. Am J Pathol 1989;134:337-44.
33. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.

CHAPTER 3.2

SOMATOSTATIN RECEPTOR SCINTIGRAPHY

IN MALIGNANT LYMPHOMAS

Abstract

Normal as well as activated lymphocytes, macrophages and leukaemic cells have previously been shown by radio receptor analysis to express receptors for somatostatin. An ^{111}In labelled somatostatin analogue was already used successfully in the visualization of a variety of neuroendocrine tumours. We investigated ten consecutive patients with malignant lymphomas (Hodgkin's disease and non-Hodgkin's lymphomas). In all patients the lymphoma deposits could be visualized with somatostatin receptor imaging. In four patients additional tumour localizations were observed as compared to the results of combined physical and radiological examinations (computed tomography and ultrasonography). In three cases tissue biopsies were taken and confirmed by autoradiography to be somatostatin receptor positive. These data indicate that malignant lymphomas may express somatostatin receptors in sufficient numbers and density to allow in vivo tumour visualization with a radiolabelled somatostatin analogue.

Introduction

In the literature several reports have been published concerning the presence of somatostatin receptors on a variety of white blood cells (1-3). Receptors for somatostatin were also identified on human leukaemic cells in lymphoblastic leukaemia and in non-lymphocytic leukaemia. Most lymphocytic leukaemia cells were found to express two classes of binding sites, high and low affinity receptors, in contrast to the single class low affinity receptors in activated lymphocytes (4). Somatostatin exerts in certain conditions an inhibitory effect on lymphocyte proliferation, immunoglobulin synthesis as well as on the production of growth factors (5-11). In previous studies we have shown that the localization of a number of somatostatin receptor positive endocrine tumours like carcinoids, endocrine pancreatic tumours and paragangliomas, can be visualized after the intravenous application of a radiolabelled somatostatin analogue (12-16).

In the present study we examined somatostatin receptor imaging of tumour tissue in vivo in patients with Hodgkin's disease and non-Hodgkin's lymphomas.

Materials and methods

Patients

Ten patients with non-Hodgkin's lymphoma or Hodgkin's disease were included. The major pathological and clinical features of these cases are listed in Table 1. Patients were classified according to both the International Working Formulation system (17), and the Kiel classification (18).

Somatostatin receptor scintigraphy

For in vivo somatostatin receptor imaging [DTPA-D-Phe^1]-octreotide was obtained from Sandoz (Basel, Switzerland). The preparation of [^{111}In-DTPA-D-Phe^1]-octreotide, the dose administered (200-250 MBq), the technique of scintigraphy with the gamma camera, as well as with single photon emission computed tomography (SPECT) have been described previously (12,16). The ideal imaging time was 24 hours after administration. The scintigram was repeated at 48 hours in cases with radiopharmaceutical radioactivity in the intestinal tract. The scintigrams were scored by two investigators from the Department of Nuclear Medicine, Erasmus University Hospital Rotterdam, the Netherlands, independently of the evaluation of the clinical, pathological, radiological and haematological parameters. In normal individuals physiological accumulation of radioactivity is seen 24 hours after administration of [^{111}In-DTPA-D-Phe^1]-octreotide in the pituitary and thyroid gland, the liver, spleen, kidneys, as well as the urinary bladder. Radioactivity at other sites was considered to be suspect for the presence of somatostatin receptor positive tissue. Tumour localization in the liver was considered to be present in case of heterogenous hepatic accumulation of the radioligand.

Somatostatin receptor autoradiography

The presence of somatostatin receptors was investigated by autoradiography on 10 μm thick frozen sections of lymphoma tissue, as described in detail for various types of tumorous and non-tumorous tissues (19-22). [^{125}I-Tyr^3]-octreotide was used as radioligand, as it had been shown previously to specifically label somatostatin receptors (19).

Results

In Table 1 the results of the comparison between tumour localizations found with initial radiological investigations (chest X-ray, computed tomography (CT) and ultrasonography) and ^{111}In labelled somatostatin analogue scanning are presented in seven patients with untreated lymphomas and three patients (patient 4, 5 and 6) with recurrent lymphomas of different types.

In patient 1 mediastinal lymphomas, which had been missed on the chest X-ray and CT scan, were apparent with ^{111}In labelled somatostatin analogue scanning (Figure 1). On repeated evaluation of the CT scan mediastinal localizations of lymphoma were seen and the assessment 'negative' was revised. The latter metastasis of lymphoma tissue were later confirmed at autopsy. Autoradiographic investigation (Figure 2) also confirmed the presence of specific somatostatin receptors in the lymphoma tissue of this patient, mainly in the central lymphoblastic areas of the lymphoma. In patient 2 the deep ulcerative lesions in the lower part of the right leg were positive at somatostatin receptor imaging (Figure 3). The mediastinal lymphoma in patient 2 (Figure 4), had been missed on the X-ray and CT scans of the thorax, also after retrospective survey of the CT scan. This patient indeed developed a radiologically evident mediastinal lymphoma six months later. Unfortunately, we did not repeat somatostatin receptor scintigraphy at that stage in order to see whether a clearer visualization was present. Mediastinal involvement of lymphoma was confirmed to be present later at autopsy. Autoradiography of these lesions again demonstrated somatostatin receptors in the lymphoma tissue. Also in patients 6 and 8 additional tumour localizations were detected at scintigraphy as compared with radiological techniques (Figure 5). Additional ultrasound and/or CT examinations confirmed the presence of tumour tissue at sites with high radioactivity in both patients. Tissue biopsies at the tumour sites in patients 1, 2, and 7 confirmed the presence of lymphoma tissue, which was in all instances somatostatin receptor positive at autoradiography. Patients 9 and 10, who had Hodgkin's disease, also showed in vivo somatostatin receptor positivity of the lymphoma tissue. In patients 3, 4, 5, 7, 9, and 10 tumour localizations indicated by ultrasound and/or CT examinations completely paralleled the investigations with ^{111}In labelled somatostatin analogue. Moreover, in none of the patients the conventional staging procedure indicated lymphoma sites that had been missed by somatostatin scintigraphy.

Table 1. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe ^{1}]-octreotide in patients with malignant lymphomas.

Patient	Diagnosis	Classification*		Stage	Site of lymphoma		
					Radiology	Scintigraphy	Autoradiography
1	non-Hodgkin	(a)	centroblastic centrocytic	II	pancreas, truncus coeliacus and para-aortal	supraclavicular, mediastinal, liver hilus, pancreas, lower abdomen	positive
		(b)	diffuse, mixed small cleaved / large cell				
2	non-Hodgkin	(a)	centroblast	-	localization visible at physical examination	skin right leg, skin above liver region and mediastinal	positive
		(b)	diffuse, large cell				
3	non-Hodgkin	(a)	polymorph (immunocytoma)	I	brain	brain	not done
		(b)	diffuse, mixed small cleaved / large cell				
4	non-Hodgkin	(a)	centrocytic	II	left pleura and left rib	lung on left side	not done
		(b)	diffuse, small cleaved				
5	non-Hodgkin	(a)	lymphoplasmacytic lymphoplasmacytoid	I	right upper leg	right upper leg	not done
		(b)	small, plasmacytoid				

Continued...

Table 1. (cont.)

Patient	Diagnosis	Classification*	Stage	Site of lymphoma		
				Radiology	Scintigraphy	Autoradiography
6	non-Hodgkin	(a) centroblastic centrocytic diffuse (b) diffuse, large cell	IV	cervical, supraclavicular, right kidney, para-aortal, lung hilum left side	cervical, supraclavicular, right kidney, para-aortal, both lung hila	not done
7	non-Hodgkin	(a) anaplastic centrocytic diffuse (b) diffuse, large cell	II	submandibular, parapharyngeal right side	submandibular, supraclavicular right side	positive
8	non-Hodgkin	Lennert's lymphoma, large cell	III	stomach, truncus coeliacus, para-aortal, supraclavicular right side	neck, supraclavicular, lung hila, upper abdomen, liver hilum, para-aortal	not done
9	Hodgkin	nodular sclerosis	II	mediastinal, retro- and parasternal, paratracheal	mediastinal, supraclavicular	not done
10	Hodgkin	nodular sclerosis	I	supraclavicular	supraclavicular	not done

* (a) Kiel classification; (b) International Working Formulation system

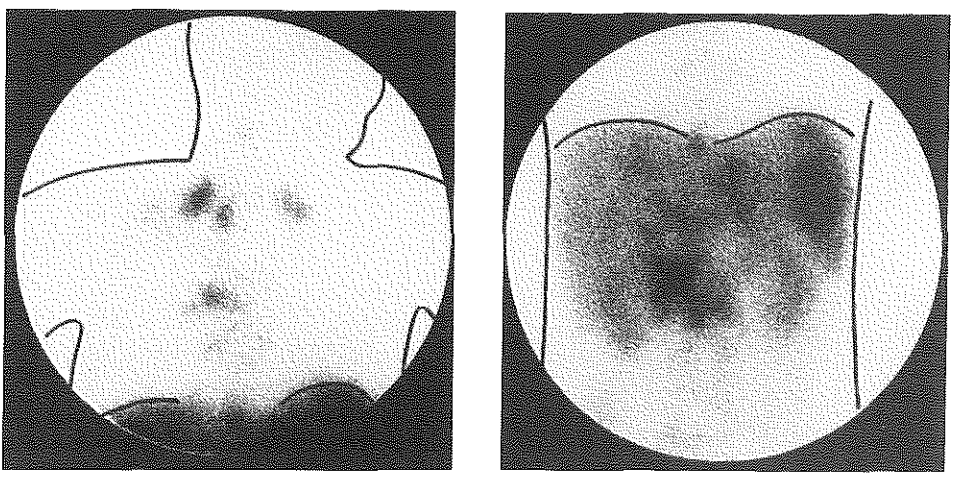

Figure 1. Visualization of the mediastinal, and supraclavicular lymph nodes (A), and lymphoma localizations in the pancreas and liver hilus (B) in a non-Hodgkin's lymphoma (patient 1, see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior views, 48 hours after injection).

Discussion

In the present study we report the presence of somatostatin receptors on the lymphoma tissue of a variety of types of non-Hodgkin's as well as Hodgkin's lymphomas. The localization of somatostatin receptors is mainly in the lymphoblastic areas of the lymphomas, representing the most active central part of these tumours. The affinity and/or the density of somatostatin receptors on these previously untreated or recurrent lymphomas was so high that in vivo visualization of these receptors with the ^{111}In labelled somatostatin analogue was possible. In previous studies the use of this scintigraphic technique was shown to be successful in visualizing endocrine tumours like carcinoids, endocrine pancreatic tumours and paragangliomas, which contain high numbers of somatostatin receptors (13,14,22).

In the present study the lymphoma tissue of the ten patients investigated could be visualized. In four of these patients additional tumour localizations, which had been missed initially at physical examination and routine radiological and ultrasound examination, were detected by somatostatin

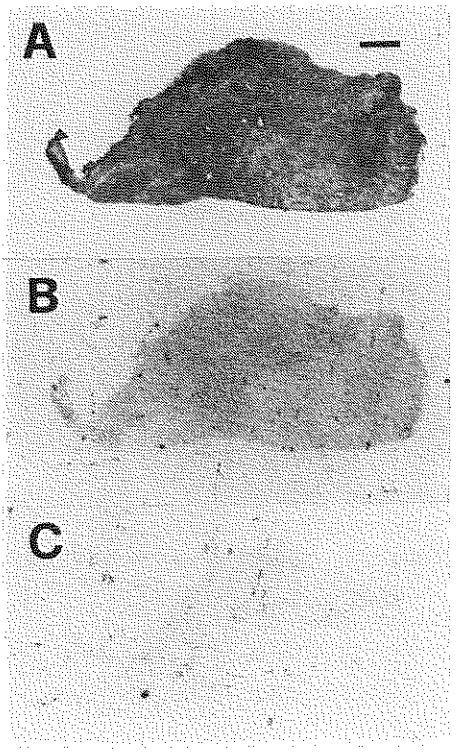

Figure 2. Visualization of somatostatin receptors in a supraclavicular lymphoma of a patient with non-Hodgkin's lymphoma (patient 1, see Table 1). (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide in the presence of 1 μM [Tyr^3]-octreotide. Bar represents 1 mm.

receptor scintigraphy. Visualization of tumour tissue by somatostatin receptor scintigraphy is not specific for lymphomas. A variety of granulomatous diseases like sarcoidosis, aspergillosis, and tuberculosis can also be visualized. The additional sites of radioactivity in our patients indeed represented lymphoma tissue, as was confirmed by biopsies or post-mortem pathology. In six patients the results of radiological examinations and ^{111}In labelled somatostatin receptor scintigraphy were parallel, i.e. the same tumour localizations were visualized. Therefore no false negative results were obtained in this group of lymphoma patients studied.

Previous studies have shown that monocytes and lymphocytes as well as lymphoblasts express somatostatin receptors (1,3-5). Functional studies indicate that somatostatin may inhibit [^{3}H]-thymidine incorporation of phytohaemagglutinin and concanavalin A stimulated lymphocytes in vitro

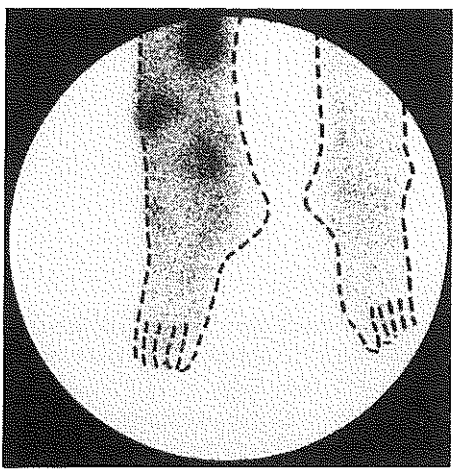

Figure 3. Somatostatin receptor positive lymphoma tissue in the lower right leg of patient 2 (see Table 1), visualized with ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

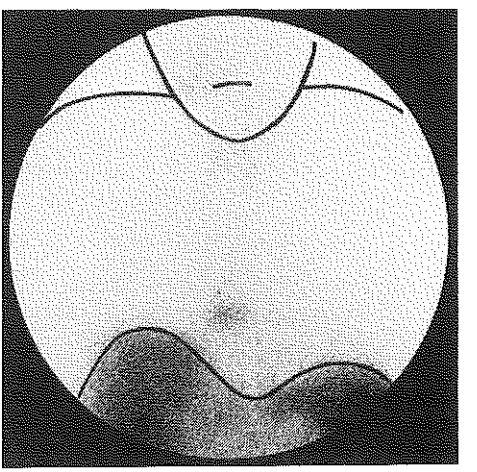

Figure 4. Visualization of the somatostatin receptor positive mediastinal lymphoma in patient 2 (see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 48 hours after injection).

(5-9,11). Somatostatin may also suppress the synthesis of immunoglobulins in an IgA secreting plasmacytoma cell line (10). Our current observations represent evidence of the presence of somatostatin receptors in human lymphomas of variable histology.

The scintigraphic technique described in this study is an easy, harmless procedure which might add to optimize staging procedures in this type of patient. At present staging in non-Hodgkin's lymphoma has become less important as more intensive chemotherapy regimens are used for most grades of disease. Possibly, this form of imaging could also be of benefit when assessing patients for autologous bone marrow transplantation, where the best results are seen when the patient is in complete remission. Further studies are obviously needed to define more precisely the value and limitations of somatostatin scintigraphy in lymphoma patients. For instance, the physiological uptake of radioactivity in the liver and spleen after intravenous administration of ^{111}In labelled somatostatin analogue might hamper optimal tumour detection in these areas, even if a subtraction

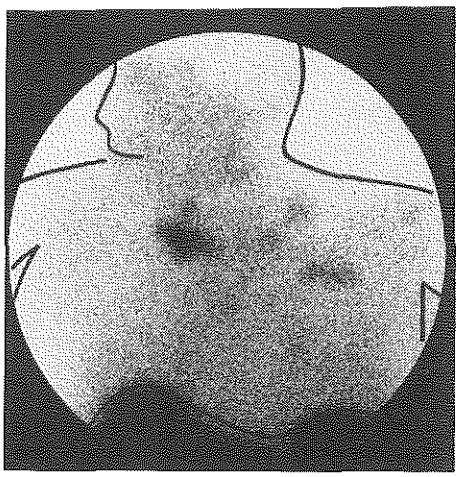

Figure 5. Visualization of the somatostatin receptor positive supraclavicular lymph nodes in patient 8 (see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

technique with [^{99m}Tc]-colloid scanning is used. Apart from a role of ^{111}In labelled somatostatin analogue scintigraphy in lymphoma staging, it is tempting to hypothesize about the potential use of such a somatostatin analogue coupled to a ß emitting radionuclide in radiotherapy of these diseases.

References

1. Bhathena SJ, Louie J, Schechter GP, Redman RS, Wahl L, Recant L. Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 1981;30:127-31.
2. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987;1:173-84.
3. Sreedharan SP, Kodama KT, Peterson KE, Goetzl EJ. Distinct subsets of somatostatin receptors on cultured human lymphocytes. J Biol Chemistry 1989;264:949-52.
4. Hiruma K, Koike T, Nakamura H, Sumida T, Maeda T, Tomioka H, Yoshida S, Fujita T. Somatostatin receptors on human lymphocytes and leukaemia cells. Immunology 1990;71:480-5.
5. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T-lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.
6. Mascardo RN, Barton RW, Sherline P. Somatostatin has an antiproliferative effect on concanavalin A-activated rat thymocytes. Clin Immunol Immunopathol 1984;33:131-8.

7. Pawlikowski M, Stepien H, Kunert-Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985;129:52-5.
8. Stanisz AM, Scicchitano R, Payan DG, Bienenstock J. In vitro studies of immunoregulation by substance P and somatostatin. Ann N Y Acad Sci 1987;496:217-25.
9. Pawlikowski M, Stepien H, Kunert-Radek J, Zelazowski P, Schally AV. Immunomodulatory action of somatostatin. Ann N Y Acad Sci 1987;496:233-9.
10. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. J Immunol 1988;141:937-41.
11. Malec P, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmocology 1989;17:45-9.
12. Krenning EP, Bakker WH, Breeman WAP, Koper JW, Kooij PPM, Ausema L, Lameris JS, Reubi JC, Lamberts SWJ. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989;1:242-4.
13. Lamberts SWJ, Hofland LJ, van Koetsveld PM, Reubi JC, Bruining HA, Bakker WH, Krenning EP. Parallel in vivo and in vitro detection of functional somatostatin receptors in human endocrine pancreatic tumors. Consequences with regard to diagnosis, localization and therapy. J Clin Endocrinol Metab 1990;71:566-74.
14. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.
15. Lamberts SWJ, Krenning EP, Klijn JGM, Reubi JC. Clinical applications of somatostatin analogues. Trends Endocrinol Metab 1990;139-44.
16. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, Lamberts SWJ, Visser TJ, Krenning EP. [^{111}In-DTPA-D-Phe^{1}]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991;49:1583-91.
17. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. Cancer 1982;49:2112-35.
18. Stansfeld AG, Diebold J, Noel H, Kapancy Y, Rilke F, Kelenyi G, Sundstrom C, Lennert K, van Unnik JAM, Mioduszewska O, Wright DH. Updated Kiel classification for lymphomas [letter]. Lancet 1988;1:292-3.
19. Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
20. Reubi JC, Maurer R. Autoradiographic mapping of somatostatin receptors in the rat central nervous system and pituitary. Neuroscience 1985;15:1183-93.
21. Reubi JC, Maurer R, Klijn JGM, Stefanko SZ, Foekens JA, Blaauw G, Blankenstein MA, Lamberts SWJ. High incidence of somatostatin receptors in human meningiomas: biochemical characterization. J Clin Endocrinol Metab 1986;63:433-8.
22. Reubi JC, Maurer R, von Werder K, Torhorst J, Klijn JGM, Lamberts SWJ. Somatostatin receptors in human endocrine tumors. Cancer Res 1987;47:551-8.

CHAPTER 3.3

SOMATOSTATIN RECEPTORS IN MALIGNANT LYMPHOMAS

Somatostatin receptors in malignant lymphomas

Somatostatin receptors have been identified in Hodgkin's disease and non-Hodgkin's lymphomas as described in Chapter 3.1 and Chapter 3.2. In vitro autoradiography demonstrated somatostatin receptors to be predominantly present in the lymphoblastic areas of the lymphomas, which represent the most active central part of these tumours. Unfortunately, somatostatin receptor autoradiography is not suitable for the identification of the somatostatin receptor expressing single cell types. Moreover, no reproducible technique is available at the moment to analyze single cells. In our studies fluorescent labelled somatostatin analogues did not sufficiently identify somatostatin receptors on mononuclear cells. Even on endocrine cells the receptor density was too low to allow the successful application of this technique at the level of single cells (data not shown). These observations are in contrast with the findings presented by others (1-3).

Recently, expression of messenger ribonucleic acid (mRNA) for somatostatin receptors has been found in two patients with non-Hodgkin's lymphoma (receptor subtypes 2 and 3) and one patient with Hodgkin's disease (receptor subtype 2) (4). Besides, somatostatin receptor expression has also been described on the veins in the immediate vicinity of non-Hodgkin's lymphoma, even when tumour cells lacked somatostatin receptors. This suggests that somatostatin receptors may also have a role in the haemodynamic tumour-host interactions (4). In low grade B cell lymphomas with follicular histology a non-homogeneous distribution of somatostatin receptors was observed, comparable with the observation in normal gastrointestinal associated lymphoid tissue. In most high grade lymphomas of the B cell type the homogeneous distribution of somatostatin receptors reflected the diffuse histopathology of these tumours.

Use of somatostatin receptor scintigraphy in staging of lymphomas

The presence of somatostatin receptors on malignant cells of lymphoid origin allows the in vivo visualization of malignant lymphomas by somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^{1}]-octreotide as radioligand (Figure 1 and 2).

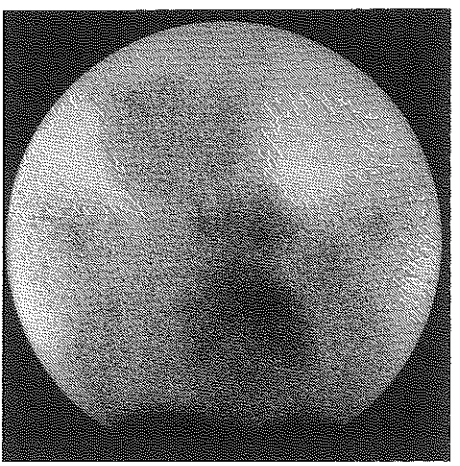

Figure 1. Visualization of mediastinal and supraclavicular lymphoma localizations in a patient with Hodgkin's disease by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

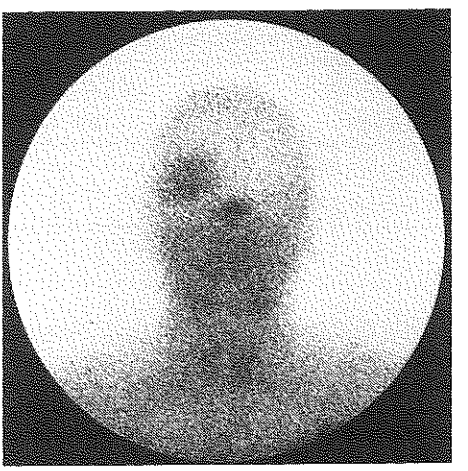

Figure 2. Visualization of an intracranial non-Hodgkin's lymphoma in a patient with the acquired immunodeficiency syndrome (AIDS) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

Recently, 40 consecutive, previously untreated, patients with histologically proven Hodgkin's disease were studied (5,6). The results of the initial conventional staging methods, i.e. physical examination, computed tomography of the chest and abdomen, bone marrow biopsy and lymphangiography, were blindly compared with those of somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^1]-octreotide (Table 1). In 39 patients (97.5%) somatostatin receptor positive lesions were detected at scintigraphy. In 17 patients somatostatin receptor scintigraphy was in agreement with the conventional staging methods. In 18 patients more lesions were found with scintigraphy, in five patients more lesions were found with the conventional staging methods. When somatostatin receptor scintigraphy was added to the conventional staging methods, the clinical stage was upgraded in six patients with Hodgkin's disease (Table 2).

Somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^1]-octreotide, was performed in 62 consecutive, previously untreated, patients with histologically proven non-Hodgkin's lymphoma (5,6). Of these, 53 (85%) were positive at scintigraphy. In 31 patients concordance between the results of conventional

Table 1. Comparison of somatostatin receptor scintigraphy (SRS) and conventional staging methods (CSM) in patients with Hodgkin's disease or non-Hodgkin's lymphoma (5,6).

Diagnosis	SRS in agreement with CSM	SRS superior to CSM	SRS inferior to CSM	Total
Hodgkin's disease	17	18	5	40
Non-Hodgkin's lymphoma	31	17	14	62

Table 2. Change in clinical stage in Hodgkin's disease and non-Hodgkin's lymphoma with ^{111}In labelled somatostatin analogue scintigraphy (5,6).

Diagnosis	Total number of patients	Upgrade
Hodgkin's disease	40	6
Non-Hodgkin's lymphoma	62	13

staging methods and somatostatin receptor scintigraphy was found (Table 1). In 17 patients somatostatin receptor scintigraphy disclosed additional lymphoma localizations. Somatostatin receptor scintigraphy failed to demonstrate selected sites of non-Hodgkin's lymphoma in five patients. When somatostatin receptor scintigraphy and the conventional staging methods were combined, the clinical stage was upgraded in 13 patients with non-Hodgkin's lymphoma (Table 2).

Several other studies, using ^{111}In labelled somatostatin analogue scintigraphy, reported lower sensitivity rates for the detection of malignant lymphomas than the rates we found (7-11) (Table 3). One explanation for the difference in the observed sensitivity rates may be a different composition of the patient groups. For example, in the study of Bong et al., the exclusion of patients with low grade non-Hodgkin's lymphoma or chronic lymphocytic leukaemia resulted in a higher sensitivity (72% vs. 39%). Moreover, the specific activity of the ligand used was lower than in our studies (100-200 MBq/10 μg vs. 200-250 MBq/10 μg). Differences in sensitivity rates may also be explained by differences in the preset counting time and the specifications of the gamma camera used.

Unfortunately, these data were not available from all studies. Finally, in our studies we consequently performed single photon emission computed tomography (SPECT) of the abdomen. In none of the other studies SPECT

Table 3. Reported sensitivity rates of ^{111}In labelled somatostatin analogue scintigraphy in Hodgkin's disease and non-Hodgkin's lymphoma (7-11).

	Sensitivity	
Authors	Hodgkin's disease	Non-Hodgkin's lymphoma
Bong et al. (8)*	92%	39%
Sarda et al. (10)*	58%**	58%**
Bares et al. (7)*	78%	63%
Ivancevic et al. (11)	86%	82%
Lipp et al. (9)	70%	35%

* Sensitivity based on the number of lesions detected.

** Combined sensitivity. No separate data for Hodgkin's disease and non-Hodgkin's lymphoma were reported.

imaging of the abdomen was performed consequently. Therefore, the low sensitivity rates reported in these studies may have been caused by the failure to detect tumour localizations in the abdominal region (7). In our opinion, SPECT imaging, using 200-250 MBq of [^{111}In-DTPA-D-Phe^{1}]-octreotide, is an important additional technique in the detection of abdominal tumour localizations in patients with Hodgkin's disease or non-Hodgkin's lymphoma.

Octreotide does not specifically visualize lymphoma tissue. A variety of granulomatous diseases like sarcoidosis, aspergillosis and tuberculosis can also be visualized. The additional sites of accumulation of radioactivity in our lymphoma patients, however, did represent malignant lymphoma tissue, as was investigated in almost all patients by biopsies or post-mortem examination. The in vitro identification of somatostatin receptors in biopsies of the malignant lymph nodes confirmed that the activity seen in vivo actually represented somatostatin receptor positive tumour tissue (12).

Somatostatin receptor scintigraphy is an easy and harmless procedure that may contribute to the optimization of staging procedures in patients with malignant lymphoma's. It offers a number of distinct advantages when compared with conventional staging methods. Firstly, the whole body is imaged, enabling the detection of tumour localizations in areas not under clinical suspicion, and herewith the accurate documentation of the extent of the disease. Secondly, scintigraphy may distinguish malignant disease from non-malignant disease.

Table 4. Responses to octreotide treatment in lymphoproliferative disorders (13).

Diagnosis	Partial remission	Stable	Progression
Low grade non-Hodgkin's lymphoma	10	14	4
Chronic lymphocytic leukaemia	0	15	4
Cutaneous T cell lymphoma	4	2	3

This may be of value in the management of residual masses, for example with the notoriously difficult mediastinal localizations. Somatostatin receptor scintigraphy may also be useful in the avoidance of overtreatment in already cured patients. A shortcoming of this scintigraphic technique is the uptake of radioactivity in the liver and spleen after intravenous administration of [^{111}In-DTPA-D-Phe^{1}]-octreotide, which may hamper optimal tumour detection in these areas, even if a subtraction technique with [^{99m}Tc]-colloid scanning is used. Therefore, SPECT imaging is an important additional technique in the detection of tumour localizations in the upper abdomen, as mentioned above.

Further studies are obviously needed to define more precisely the possibilities and limitations of [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy in patients with malignant lymphomas.

Octreotide in the treatment of malignant lymphomas

Apart from the role of octreotide in the staging of disease, evaluation of the somatostatin receptor status may be of value in the treatment of patients with malignant lymphomas with somatostatin analogues. The efficacy of the somatostatin analogue octreotide (Sandostatin®), 150 μg tid, has been demonstrated in 56 patients with lymphoproliferative disorders (Table 4) (13). Partial remissions were found in patients with low grade non-Hodgkin's lymphoma and cutaneous T cell lymphoma. No remissions were found in patients with chronic lymphocytic leukaemia, which is in agreement with the observation that somatostatin receptor scintigraphy is negative in this disease (unpublished data). The somatostatin receptor status of patients with malignant lymphomas will become particularly important when somatostatin analogues coupled to a ß emitting radionuclide will become available for radiotherapy of these diseases.

References

1. Payan DG, Brewster DR, Missirian-Bastian A, Goetzl EJ. Substance P recognition by a subset of human T lymphocytes. J Clin Invest 1984;74:1532-9.
2. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987;1:173-83.
3. Hiruma K, Koike T, Nakamura H, Sumida T, Maeda T, Tomioka H, Yoshida S, Fujita T. Somatostatin receptors on human lymphocytes and leukaemia cells. Immunology 1990;71:480-5.
4. Reubi JC, Schaer JC, Waser B, Mengod G. Expression and localization of somatostatin receptor SSTR1, SSTR2, and SSTR3 messenger RNAs in primary human tumors using in situ hybridization. Cancer Res 1994;54:3455-9.
5. van den Anker-Lugtenburg PJ, Krenning EP, Oei HY, Gerrits CJH, van Hagen PM, Reubi JC, Lamberts SWJ, Löwenberg B. The role of somatostatin receptor scintigraphy (SRS) in the initial staging of malignant lymphoma. [Abstract] J Nucl Med 1994;35:132.
6. van Hagen PM, van den Anker-Lugtenburg PJ, Reubi JC, Lamberts SWJ, Krenning EP. The role of octreotide scintigraphy in malignant lymphomas, granulomatous diseases and rheumatoid arthritis. Handbook of targeted delivery of imaging agents. Torchilin VP, editor. CRC Press, Inc., 1995:631-44.
7. Bares R, Galonska P, Dempke W, Handt S, Bull U, Osieka R. Somatostatin receptor scintigraphy in malignant lymphoma: first results and comparison with glucose metabolism measured by positron-emission tomography. Horm Metab Res Suppl 1993;27:56-8.
8. Bong SB, VanderLaan JG, Louwes H, Schuurman JJ. Clinical experience with somatostatin receptor imaging in lymhoma. Semin Oncol 1994;21:46-50.
9. Lipp RW, Silly H, Ranner G, Dobnig H, Passath A, Leb G, Krejs GJ. Radiolabeled octreotide for the demonstration of somatostatin receptors in malignant lymphoma and lymphadenopathy. J Nucl Med 1995;36:13-8.
10. Sarda L, Duet M, Zini JM, Berolatti B, Benelhadj S, Tobelem G, Mundler O. Interest of ^{111}In-pentetreotide scintigraphy in the staging and follow-up of malignant lymphomas. [Abstract] J Nucl Med 1995;36:195.
11. Ivancevic V, Nauck C, Wörmann B, Sandrock D, Hiddemann W, Munz DL, Emrich D. Is somatostatin receptor scintigraphy useful in staging Hodgkin- and non-Hodgkin lymphomas? [Abstract] J Nucl Med 1995;36:199.
12. van Hagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY, Löwenberg B, Lamberts SWJ. Somatostatin analogue scintigraphy of malignant lymphomas. Br J Haematol 1993;83:75-9.
13. Witzig TE, Letendre L, Gerstner J, Schroeder G, Maillaird JA, Colon-Otero G, Marschke RF, Windschitl HE. Evaluation of a somatostatin analog in the treatment of lymphoproliferative disorders: results of a phase II North Central Cancer Treatment Group trial. J Clin Oncol 1995;13:2012-5.

CHAPTER 4

SOMATOSTATIN RECEPTORS IN GRANULOMATOUS DISEASES

4.1 Somatostatin receptors in granulomatous diseases

4.2 Somatostatin receptor scintigraphy in granulomatous diseases

CHAPTER 4.1

SOMATOSTATIN RECEPTORS IN GRANULOMATOUS DISEASES

Granulomatous inflammation

Granulomatous inflammation is manifested in chronic inflammatory diseases that often result in tissue destruction and end-stage fibrosis. The common histological feature of granulomatous inflammations is the infiltration of mononuclear leucocytes. A variety of granulomatous diseases are caused by infectious agents (tuberculosis, histoplasmosis) or non-infectious agents (silicosis, berylliosis). Others are of unknown origin (sarcoidosis, Wegener's granulomatosis). Within the group of granulomatous diseases there is heterogeneity with regard to the cellular infiltrate and the intensity of specific lesions. Granulomatous inflammations can roughly be classified as either a hypersensitivity type response (immunological) or a foreign body type response (non-immunological). This classification is based on the degree of antigen specific lymphocytes in the developing lesion. In both granuloma types, macrophages and their derivatives, epithelioid cells and giant cells, are constant components of the lesion.

The pathogenetic mechanism of granuloma formation starts with the presentation of an antigen by an antigen presenting cell in concordance with HLA class II molecules. Antigen presentation activates T helper 1 cells to produce interferon γ and interleukin 2. Interferon γ is an important cytokine in the induction of granuloma formation, because it enhances the expression of HLA class II molecules, Macrophages are producers of the lymphokines interleukin 1 and tumour necrosis factor α, which have overlapping and related biological activities, but are encoded by unique gene sequences. In particular, interleukin 1 can induce the production of interleukin 2 by T lymphocytes. Interleukin 1 dependent interleukin 2 stimulation has been shown to be an important signal for growth and clonal expansion of effector T cell populations. Experimental data suggest that interleukin 1 plays a key role in the development of granulomatous inflammations (1). Granuloma macrophages are able to produce tumour necrosis factor α as well. This cytokine has a role in the maintenance of established granuloma lesions.

Somatostatin receptors in granulomas

A well known model for granulomas of the hypersensitivity type response is the *Schistosoma mansoni* egg induced antigen specific granuloma (2). This delayed type hypersensitivity reaction is a rapidly progressing, florid, granulomatous response, characterized by activated macrophages, sensitized T lymphocytes and antibody secreting B cells. The *Schistosoma mansoni* egg induced granuloma model was studied extensively in relation to neuropeptides and neuropeptide receptor expression (3-7). T lymphocytes, isolated from hepatic granulomas and predominantly CD4 positive, have a high affinity for somatostatin 14 (K_d = 4.3 nM) and octreotide (K_d = 0.1 nM) (8). Both somatostatin 14 and octreotide suppress interferon γ synthesis by stimulated CD4 positive lymphocytes (8). Further, somatostatin 14 reduces the production of IgG2 by granuloma B cells, probably by decreasing the secretion of interferon γ (9). Continuous infusion with octreotide was found to decrease the hepatic granuloma size in vivo (10). Granuloma macrophages secrete somatostatin 14. This may be a feedback counter mechanism in the regulation of the granulomatous inflammatory response (11).

Recently, messenger ribonucleic acid (mRNA) for somatostatin receptor subtype 2 was isolated from hepatic murine granulomas by using the polymerase chain reaction technique. Also, purified CD4 positive granuloma T cells and granuloma T cell lines were shown to contain mRNA for the somatostatin receptor subtype 2 (12).

Our results on somatostatin receptor expression in human granulomatous diseases are presented in Chapter 4.2.

References

1. Kunkel SL, Chensue SW, Strieter RM, Lynch JP, Remick DG. Cellular and molecular aspects of granulomatous inflammation. [Review] Am J Respir Cell Mol Biol 1989;1:439-47.
2. Moore DL, Grove DI, Warren KS. The *Schistosoma mansoni* egg granuloma: quantitation of cell populations. J Pathol 1977;121:41-50.
3. Weinstock JV, Blum AM, Walder J, Walder R. Eosinophils from granulomas in murine schistosomiasis mansoni produce substance P. J Immunol 1988;141:961-6.
4. Weinstock JV, Blum AM. Tachykinin production in granulomas of murine schistosomiasis mansoni. J Immunol 1989;142:3256-61.

5. Weinstock JV, Blum AM. Detection of vasoactive intestinal peptide and localization of its mRNA within granulomas of murine schistosomiasis. Cell Immunol 1990;125:291-300.
6. Weinstock JV. Production of neuropeptides by inflammatory cells within the granulomas of murine schistosomiasis mansoni. [Review] Eur J Clin Invest 1991;21:145-53.
7. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. Clin Immunol Immunopathol 1992;64:17-22.
8. Blum AM, Metwali A, Mathew RC, Cook G, Elliott D, Weinstock JV. Granuloma T lymphocytes in murine schistosomiasis mansoni have somatostatin receptors and respond to somatostatin with decreased IFN-gamma secretion. J Immunol 1992;149:3621-6.
9. Blum AM, Metwali A, Mathew RC, Elliott D, Weinstock JV. Substance P and somatostatin can modulate the amount of IgG2a secreted in response to schistosome egg antigens in murine schistosomiasis mansoni. J Immunol 1993;151:6994-7004.
10. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. [Review] Clin Immunol Immunopathol 1992;64:17-22.
11. Weinstock JV, Blum AM, Malloy T. Macrophages within the granulomas of murine *Schistosoma mansoni* are a source of a somatostatin 1-14-like molecule. Cell Immunol 1990;131:381-90.
12. Elliott DE, Metwali A, Blum AM, Sandor M, Lynch R, Weinstock JV. T lymphocytes isolated from the hepatic granulomas of schistosome-infected mice express somatostatin receptor subtype II (SSTR2) messenger RNA. J Immunol 1994;153:1180-6.

CHAPTER 4.2

SOMATOSTATIN RECEPTOR SCINTIGRAPHY IN GRANULOMATOUS DISEASES

Abstract

Normal as well as activated lymphocytes and macrophages have previously been shown by radioreceptor analysis to express somatostatin receptors. The somatostatin analogue [^{111}In-DTPA-D-Phe^{1}]-octreotide has already been used successfully in the visualization of a variety of neuroendocrine tumours and malignant lymphomas. In the present study 20 consecutive patients are investigated; 12 patients with sarcoidosis, one patient with both sarcoidosis and aspergillosis, four patients with tuberculosis and three patients with Wegener's granulomatosis. For in vivo somatostatin receptor imaging, total body scintigraphy was performed 24 and 48 hours after the administration of [^{111}In-DTPA-D-Phe^{1}]-octreotide. Granuloma localizations could be visualized in all patients studied; additional sites were found in nine patients with sarcoidosis and in two patients with tuberculosis. In vitro autoradiography of fresh tissue biopsies, using the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide, showed binding at sites that were microscopically identified as granulomatous inflammation. These observations demonstrate the expression of somatostatin receptors by human granulomas. This scintigraphy procedure may contribute to a more precise staging and evaluation of granulomatous diseases. More importantly, it may be a sensitive indicator of the efficacy of glucocorticoid and/or immunosuppressive therapy.

Introduction

Granulomas may be defined as small collections of modified macrophages (epithelioid cells, multinucleated giant cells) and lymphocytes, but fibroblasts and granulocytes can also be present. Aggregated granulomas often result in pseudotumorous lesions. In more advanced stages, they may become enclosed by fibrous tissue and eventually be replaced by hyaline scars. Granulomas are manifestations of the response to a variety of antigens. They occur in mycobacterial, parasitic, fungal and other infectious diseases, as a reaction to non-infectious poorly digestible agents, and in diseases of obscure origin such as sarcoidosis, Crohn's disease and Wegener's granulomatosis.

Recently, we developed a technique in which the administration of radionuclide labelled somatostatin analogue successfully visualizes endocrine tumours and malignant lymphomas (1-3). Experimental murine *Schistosoma mansoni* induced

granulomas express somatostatin receptors and synthesize somatostatin as well (4-6). This observation led to the present study, in which we investigated the value of [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy in patients with sarcoidosis (one of whom also had aspergillosis), tuberculosis or Wegener's granulomatosis.

Materials and methods

Patients

Twenty consecutive patients were included; 13 patients with sarcoidosis, one of them having aspergillosis as well, four patients with active tuberculosis, and three patients with Wegener's granulomatosis. The diagnosis of sarcoidosis was based on the appearance of hilar lymphadenopathy, the presence of an alveolitis, as demonstrated by an increased percentage CD3 positive lymphocytes (>40%) and an increased CD4/CD8 ratio (>4) in the bronchoalveolar fluid, and the presence of epithelioid cells in transbroncheal biopsies. In six patients the diagnosis was based on biopsies taken from affected bones (patients 2 and 4), lymph nodes (patients 7,8 and 11), and liver (patients 4 and 10). The presence of aspergillosis infection of the lung in one patient and of active pulmonary tuberculosis in four patients was diagnosed on the basis of cultures of the sputum as well as direct microscopic investigation using special stains. The diagnosis Wegener's granulomatosis was based on the clinical findings, the demonstration of necrotizing granulomatous vasculitis in biopsies of the upper respiratory tract, and the presence of antineutrophil cytoplasmic antibodies.

Somatostatin receptor scintigraphy

The somatostatin analogue [DTPA-D-Phe^{1}]-octreotide was obtained from Mallinckrodt (Petten, the Netherlands). [DTPA-D-Phe^{1}]-octreotide was coupled to ^{111}In as described previously (2). [^{111}In-DTPA-D-Phe^{1}]-octreotide is excreted mainly via the kidneys, 90% of the dose being present in the urine 24 hours after injection. Because of its relatively long effective half-life, [^{111}In-DTPA-D-Phe^{1}]-octreotide is a radionuclide coupled somatostatin analogue that can be used to visualize somatostatin receptor bearing tumours and inflammation localizations efficiently after 24 and 48 hours, when the interfering background radioactivity is minimized by renal clearance (2,7,8). [^{111}In-DTPA-D-Phe^{1}]-octreotide (200-250 MBq) was injected and planar images were obtained with a large-field-of-view gamma camera (Counterbalance 3700 and ROTA II,

Siemens Gammasonics, Erlangen, Germany), equipped with a medium energy collimator. Static images were obtained 24 and 48 hours after injection of [^{111}In-DTPA-D-Phe^{1}]-octreotide, because of the low background radioactivity at that time. Preset counts for images obtained 24 hours after injection of the ^{111}In labelled octreotide, was 300,000 for the head and neck, and 500,000 for the remainder of the body; the preset counting time for images obtained 48 hours after injection of [^{111}In-DTPA-D-Phe^{1}]-octreotide, was 15 minutes (8). To define the granulomas as visualized during this scintigraphic procedure, we used a simple yes-or-no system. As a rule, and especially in the abdomen, accumulation of radioactivity at an abnormal site was considered to represent somatostatin receptor binding only if it was present on the scintigrams of both standard imaging time points. The scintigrams of all 20 patients were studied independently by two investigators, without knowing the patient's identity, medical history, or outcomes of other investigations. In order to compare the number of lesions demonstrated with conventional imaging techniques with those visualized during [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy, the images were divided in parts easily recognizable on scintigrams, i.e. the head and neck, chest, upper abdomen, lower abdomen (division line: lower edges of the kidneys), and extremities.

Somatostatin receptor autoradiography

The presence of somatostatin receptors in granuloma tissue was investigated by autoradiography on 10 μM thick frozen sections of tissue. [^{125}I-Tyr^{3}]-octreotide, which has previously been show to specifically label somatostatin receptors, was used as radioligand. This technique has been described in detail for various types of tumorous and non-tumorous tissues (9-12).

Results

After intravenous injection of [^{111}In-DTPA-D-Phe^{1}]-octreotide, physiological uptake of radioactivity was seen in all patients in the pituitary gland, the thyroid gland, the liver, the spleen, the kidneys and the bladder, as described previously (2,7,8).

The comparison of tumour localizations found with conventional imaging techniques and [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy is presented in Table 1. Patients 1-13 had sarcoidosis. In all 13 patients the known localizations

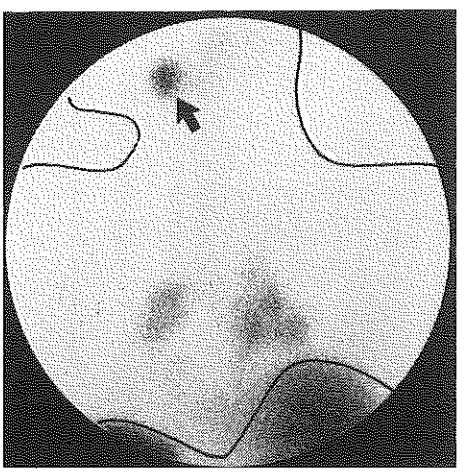

Figure 1. Bilateral uptake of [^{111}In-DTPA-D-Phe^1]-octreotide in the hila of the lungs in a patient with sarcoidosis (patient 1, see Table 1) (posterior view, 48 hours after injection). The carotid paraganglioma is indicated by an arrow.

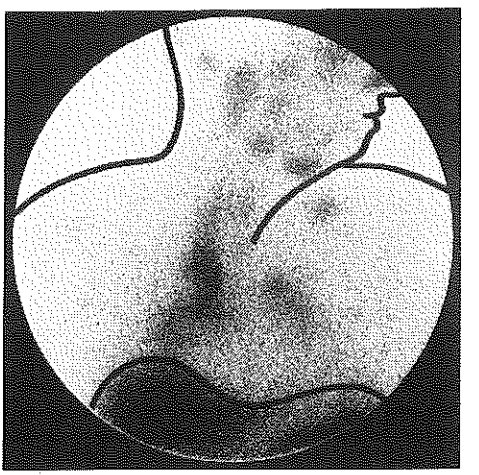

Figure 2. Visualization of the hila of the lung, supraclavicular lymph nodes and lymph nodes in the neck of a patient with sarcoidosis (patient 2, see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

of sarcoidosis could be visualized after the administration of the ^{111}In labelled octreotide, with the exception of a granuloma in a phalanx and in the liver of patient 4 and a granuloma in the left axilla of patient 7. In patient 1 a carotid paraganglioma was visualized in addition to the hila of the lung (Figure 1). A lesion in the cerebellum of patient 10, only detected by magnetic resonance imaging, could have been a granuloma, but was negative at scintigraphy. In nine of these patients, however, more granulomas were detected with whole body scintigraphy than with conventional imaging techniques (patients 2-8, 10 and 13). In all these cases the presence of 'new' granuloma sites was confirmed by additional computer tomography, ultrasound and X-ray investigations and/or biopsies of the tissue. In patient 2 the spread of granulomas in the hilar region of the lung (Figure 2) and in the bones of the hands and feet (Figure 3) was considerably more extensive than previously suspected. In patients 2, 5 and 13 additional granuloma localizations were detected in the salivary glands (normal salivary glands are not visualized with [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy). In patient 9, aspergillosis of the right lung was visualized, in addition to the affected lymph nodes in the inguinal region.

Table 1. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe ^{1}]-octreotide in patients with granulomatous diseases.

Patient	Sex*	Age	Diagnosis	Therapy	Known sites of inflammation	Scintigraphy
1	M	43	Sarcoidosis Paraganglioma	None	Lung hila	Lung hila
2	F	37	Sarcoidosis	Corticosteroids	Submandibular, supraclavicular, lung hila, liver, fingers, toes	Nose, salivary glands, neck, supraclavicular, lung hila, right knee, fingers, toes
3	M	37	Sarcoidosis	None	Left lung hilus	Lung hila
4	F	33	Sarcoidosis	Corticosteroids	Brain (parietal, right side), nose, hand, liver	Brain, lunghila, nose
5	F	18	Sarcoidosis	None	Erythema nodosum, paratracheal, carina	Right lung hilus, lacrimal glands, parotid glands
6	F	46	Sarcoidisis	None	Lung hila	Lung hila, supraclavicular, mediastinum
7	F	66	Sarcoidosis	None	Supraclavicular, right lung hilus, left axilla	Lung hila, supraclavicular
8	M	46	Sarcoidosis	Corticosteroids	Lung hila	Lung hila, supraclavicular, abdomen
9	M	40	Sarcoidosis Aspergillosis	None	Right lung, inguinal	Right lung, inguinal

Continued...

Table 1. (cont.)

Patient	Sex*	Age	Diagnosis	Therapy	Known sites of inflammation	Scintigraphy
10	F	46	Sarcoidosis	None	Cerebellum, left maxillary sinus, liver	Nose, left maxillary sinus, supraclavicular, lung hila, spleen (enlarged), liver (enlarged)
11	F	41	Sarcoidosis	None	Neck, supraclavicular, mediastinal	Neck, supraclavicular, mediastinal
12	F	52	Sarcoidosis	None	Lung hila	Lung hila
13	M	45	Sarcoidosis	None	Lungs	Lungs, parotid glands
14	M	32	Tuberculosis	None	Right lung	Right lung, left lung hilus
15	M	21	Tuberculosis	None	Left lung	Left lung
16	M	35	Tuberculosis	None	Left lung	Left lung, pericard
17	M	41	Tuberculosis	None	Lungs, peri-aortal	Lungs, upper abdomen
18	F	67	Wegener's granulomatosis	Corticosteroids Cyclophosphamide	Nose, larynx, lungs	Nose, throat, lungs
19	M	66	Wegener's granulomatosis	Corticosteroids	Nose, lungs, kidneys	Nose, lungs, kidneys
20	M	45	Wegener's granulomatosis	Corticosteroids	Nose, urethra	Nose, urethra

* M = male; F = female

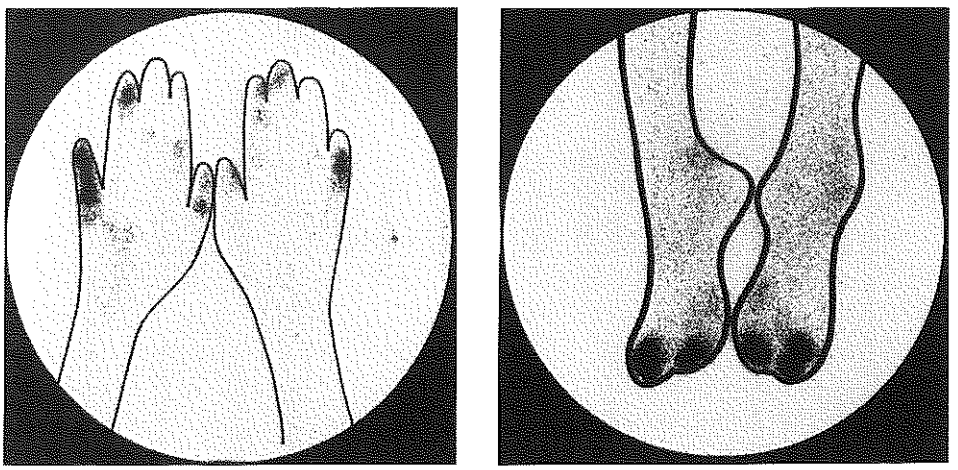

Figure 3. Bone involvement of the fingers (A) and toes (B) of a patient with sarcoidosis (patient 2, see Table 1), visualized by ^{111}In labelled somatostatin analogue scintigraphy (48 hours after injection).

Scintigraphy was repeated in two patients, one month (patient 6) and four months (patient 13) after the start of therapy with prednisone; the initial dose was 30 mg per day, and was progressively reduced over a period of one month. Both patients had responded well to this therapy, as indicated by the clinical picture (general well being, decreasing size of lymphomas by physical examination) and normalization of radiological findings. Visualization of the lesions in the hila of the lung disappeared completely in both patients. In three other patients (patient 2,4 and 8), who were also treated with prednisone therapy for one month (30 mg/day in patient 2 and 60 mg/day in patients 4 and 8) only minor changes were noted, clinically and radiologically. The granulomas of these patients remained positive at scintigraphy.

Patients 14-17 were diagnosed with active lung tuberculosis. [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy visualized the pulmonary lesions clearly. In patient 14, the right lung and a small spot in the left lung were visualized, while the chest X-ray had suggested the presence of active tuberculosis in the right lung only (Figure 4). In patient 16 we observed tuberculous involvement of the pericardium by the uptake of [^{111}In-DTPA-D-Phe^1]-octreotide in the pericardium (Figure 5).

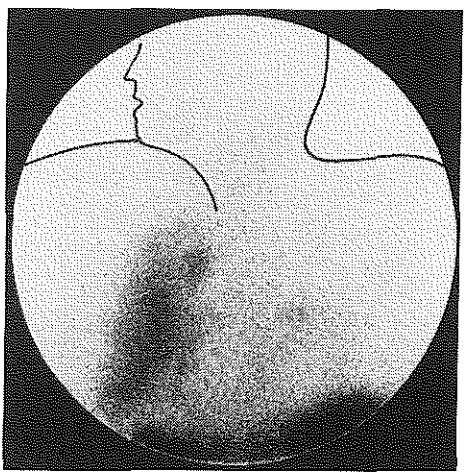

Figure 4. Imaging of the chest of a patient with tuberculosis of the right lung (patient 14, see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection). In the left lung a small spot is detectable.

Patients 18-20 had Wegener's granulomatosis. In patient 18 only a slight uptake of [^{111}In-DTPA-D-Phe^{1}]-octreotide was observed, possibly because this patient was already treated with prednisone and cyclophosphamide. Patient 20 was treated with prednisone (initial dose 60 mg/day) and azathioprine (150 mg/day). This patient did not respond very well to therapy (general well being, urethra obstruction, sinusitis and persistent positive antibodies against neutrophil cytoplasmic antigens). The [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy repeated after 2 months of therapy remained positive.

To validate the specificity of the visualization by [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy, in vitro autoradiography with [^{125}I-Tyr^{3}]-octreotide was performed on biopsies of granulomatous tissue. In vitro somatostatin receptor expression was shown in a lymph node from patient 11. The somatostatin receptors were localized in the region of epithelioid and giant cells of the granulomas. Unfortunately, we did not obtain biopsies of granulomatous tissues from the other patients included in our study. Most interestingly, however, tissue biopsies of two patients with sarcoidosis (not scanned in vivo), obtained from the Institutes of Pathology in Berne and Lucerne, Switzerland, were tested with in vitro somatostatin receptor autoradiography. The first tissue specimen, from a patient with active sarcoidosis, displayed multiple confluent typical granulomas (Figure 6). This lesion was strongly somatostatin receptor positive. The receptors were preferentially located in the areas containing epithelioid cells and

Figure 5. Three-dimensional representation of the heart in a patient with tuberculous pericarditis (patient 16, see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (24 hours after injection).

not in the zone of surrounding lymphocytes. The second patient was successfully treated with glucocorticoids. The biopsy showed complete sclerosis of the granulomatous lesions and no somatostatin receptors were detectable (Figure 7). Lymph nodes of two other patients with tuberculosis also expressed somatostatin receptors throughout the granulomatous tissue at in vitro autoradiography.

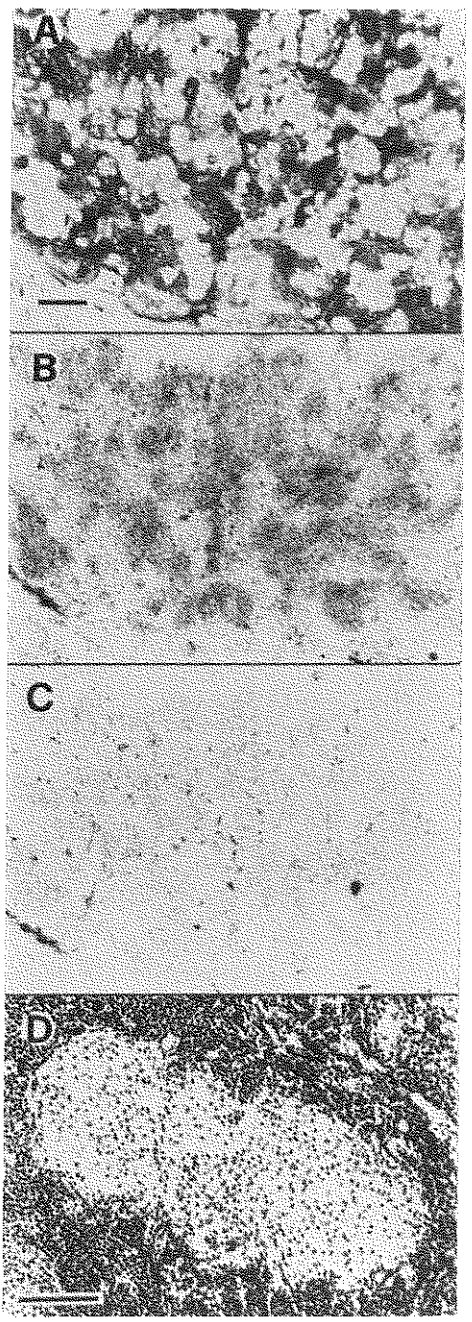

Figure 6. Visualization of somatostatin receptors in active sarcoidosis. (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. The pale granuloma tissue is labelled, but not the darker lymphocytes. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide in the presence of 1 μM [Tyr^3]-octreotide. (D) Somatostatin receptor positive granuloma shown at higher magnification. Bars represent 1 mm.

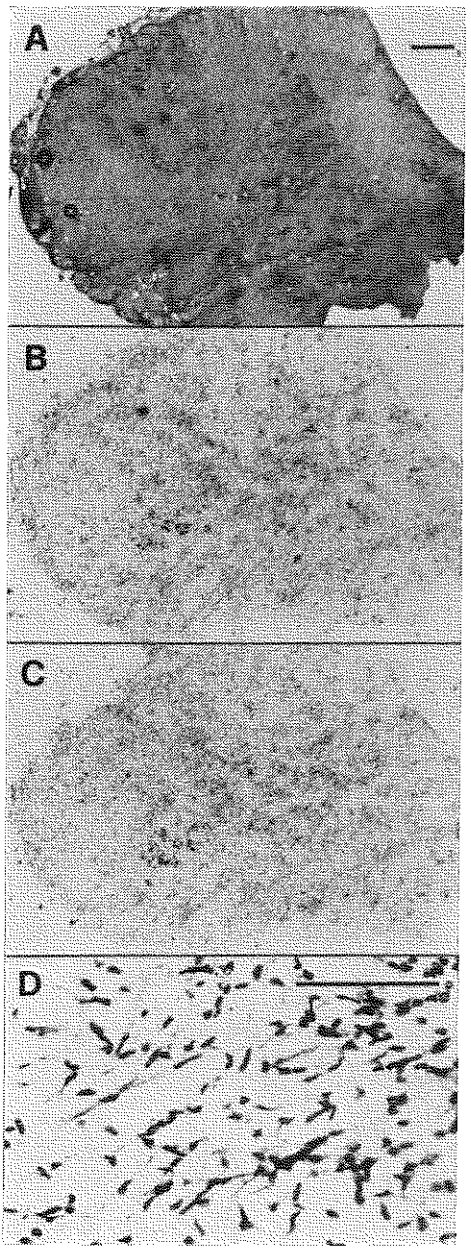

Figure 7. Absence of somatostatin receptors in a successfully treated sarcoidosis. (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^3]-octreotide. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^3]-octreotide in the presence of 1 μM [Tyr^3]-octreotide. (D) Largely sclerosed sarcoidosis shown at high magnification. Bars represent 1 mm.

Discussion

We report that granulomatous tissue of patients with active sarcoidosis, tuberculosis, aspergillosis and Wegener's disease expresses somatostatin receptors in sufficient numbers to allow in vivo visualization using an ^{111}In labelled somatostatin analogue. In all 13 patients with sarcoidosis, pulmonary as well as extra-pulmonary lesions could be visualized with [^{111}In-DTPA-D-Phe^{1}]-octreotide. In virtually all patients more granuloma localizations were detected than previously known on the basis of physical examination and routine radiological investigations. Our study also presents preliminary evidence for a role of [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy in the evaluation of the effect of glucocorticoid therapy of sarcoidosis. The scintigram became negative during successful therapy in two patients, while the granuloma sites remained positive at scintigraphy during unsuccessful therapy with prednisone in three patients. The results of the in vitro study suggest that the disappearance of somatostatin receptors in sarcoidosis lesions upon corticosteroid therapy could be due to the sclerosis of granulomas and their replacement by hyaline fibrous tissue. Granulomas in tuberculosis, aspergillosis and in Wegener's granulomatosis were also shown to be positive at [^{111}In-DTPA-D-Phe^{1}]-octreotide scintigraphy. Specific binding was observed in tuberculous granulomas at in vitro autoradiography as well. Unfortunately no tissue was obtained for in vitro autoradiography from patients with Wegener's granulomatosis. Generally, granulomas seem to express somatostatin receptors.

Experimentally, granulomas in murine schistosomiasis have been shown to express somatostatin receptors and synthesize and store somatostatin in the granules of (modified) macrophages within the granuloma. The (modified) macrophages (epithelioid cells) release somatostatin when stimulated by physiological and pharmacological agents (4-6). Synthesis of somatostatin by granuloma cells, as well as the presence of somatostatin receptors within granuloma cells, might indicate that somatostatin participates in regulating the intensity of the granulomatous response to specific microorganisms (tuberculosis, aspergillosis, schistosomiasis) or unknown stimuli (sarcoidosis). Since somatostatin seems to inhibit proliferative and other functions of lymphoid cells (13-17), it is conceivable that similar effects may take place in granulomas. In accordance with this hypothesis, it was shown that octreotide therapy decreases the intensity of the granuloma response by approximately 45% in murine schistosomiasis (6). However, granulomas are not the only lesions of the

mononuclear phagocytic and lymphoreticular system that may yield positive scintigrams. Recently, we have observed that neoplastic tissues of virtually all patients with Hodgkin's disease or non-Hodgkin's lymphoma are somatostatin receptor positive and that their localization can be shown with this technique as well (3). A possible shortcoming of somatostatin receptor visualization in granulomatous disease lies in the difficulty of detecting liver and spleen abnormalities (apparent in patient 10), while microscopically granulomas have been reported to be present in the liver in 60-90 % of all cases (18). It is conceivable that the sensitivity of this method is not sufficient for the visualization of small lesions such as those present in the liver and spleen.

In summary, we present evidence for the successful visualization of granulomatous diseases in humans, mainly of sarcoidosis, with [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy. These preliminary findings suggest that the scintigraphy procedure may contribute to a more precise staging and a better evaluation of the fairly unpredictable spontaneous course of sarcoidosis and Wegener's granulomatosis, and to an optimal choice of biopsy sites in granulomatous diseases. More importantly, it may be a sensitive indicator of the activity of disease and of the efficacy of glucocorticoid and/or immunosuppressive therapy. However, more investigations are necessary to understand the pathophysiology of somatostatin receptor expression in granulomatous diseases.

References

1. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.
2. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, Lamberts SWJ, Visser TJ, Krenning EP. [^{111}In-DTPA-D-Phe]^1-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991;49:1583-91.
3. van Hagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY, Löwenberg B, Lamberts SWJ. Somatostatin analogue scintigraphy of malignant lymphomas. Br J Haematol 1993;83:75-9.
4. Weinstock JV, Blum AM, Malloy T. Macrophages within the granulomas of murine *Schistosoma mansoni* are a source of a somatostatin 1-14-like molecule. Cell Immunol 1990; 131:381-90.
5. Weinstock JV. Production of neuropeptides by inflammatory cells within the granulomas of murine schistosomiasis mansoni. [Review] Eur J Clin Invest 1991;21:145-53.

6. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. [Review] Clin Immunol Immunopathol 1992;64:17-22.
7. Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyono-Han B, de Jong M, Kooij PPM, Bruns C, van Hagen PM, Marbach P, Visser TJ, Lamberts SWJ. In vivo application of [^{111}In-DTPA-D-Phe^1]-octreotide for detection of somatostatin receptor-positive tumors in rats. Life Sci 1991;49:1593-601.
8. Krenning EP, Bakker WH, Kooij PPM, Breeman WAP, Oei HY, de Jong M, Reubi JC, Visser TJ, Kwekkeboom DJ, Reijs AEM, van Hagen PM, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe ^{1}]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^3]-octreotide. J Nucl Med 1992;33:652-8.
9. Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
10. Reubi JC, Maurer R. Autoradiographic mapping of somatostatin receptors in the rat central nervous system and pituitary. Neuroscience 1985;15:1183-93.
11. Reubi JC, Maurer R, Klijn JGM, Stefanko SZ, Foekens JA, Blaauw G, Blankenstein MA, Lamberts SWJ. High incidence of somatostatin receptors in human meningiomas: biochemical characterization. J Clin Endocrinol Metab 1986;63:433-8.
12. Reubi JC, Maurer R, von Werder K, Torhorst J, Klijn JGM, Lamberts SWJ. Somatostatin receptors in human endocrine tumors. Cancer Res 1987;47:551-8.
13. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T-lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.
14. Pawlikowski M, Stepien H, Kunert-Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985;129:52-5.
15. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
16. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. J Immunol 1988;141:937-41.
17. Malec P, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmacology 1989;17:45-9.
18. Crystal RG. Sarcoidosis. In: Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS, editors. Harrison's principles of internal medicine. New York: McGraw-Hill, Inc. 1987:1448.

CHAPTER 5

SOMATOSTATIN RECEPTORS IN RHEUMATOID ARTHRITIS AND SJÖGREN'S SYNDROME

5.1 Somatostatin receptor scintigraphy in rheumatoid arthritis

5.2 Vascular somatostatin receptors in rheumatoid arthritis

5.3 Somatostatin receptor scintigraphy in Sjögren's syndrome

CHAPTER 5.1

SOMATOSTATIN RECEPTOR SCINTIGRAPHY IN RHEUMATOID ARTHRITIS

Abstract

In this study the expression of somatostatin receptors on synovial membranes was investigated in vivo and in vitro in patients with rheumatoid arthritis. The joints of 14 consecutive patients with active rheumatoid arthritis, four patients with severe osteoarthritis, and 30 control patients were studied. The somatostatin analogue [^{111}In-DTPA-D-Phe^{1}]-octreotide was used for in vivo somatostatin receptor scintigraphy and the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide was used for in vitro somatostatin receptor autoradiography. Seventy-six percent (220 of 290) of the painful joints and 76% (207 of 274) of the swollen joints of the patients with rheumatoid arthritis were visualized by somatostatin receptor scintigraphy. The degree of pain and swelling correlated well with positive scintigraphic findings in the joints ($p<0.0001$). In two patients with rheumatoid arthritis who underwent scintigraphy, as well as in four of five other patients, in vitro studies of the synovial membranes showed the presence of specific somatostatin receptors. In patients with osteoarthritis the uptake of radioactivity in the affected joints was significantly lower than that in patients with rheumatoid arthritis. None of the joints of the control patients demonstrated uptake of radioactivity. We conclude that somatostatin receptors are present in the synovial tissue of patients with active rheumatoid arthritis, as demonstrated by both in vivo and in vitro techniques. The potential value of somatostatin receptor scintigraphy in the clinical evaluation of patients with active rheumatoid arthritis is presently unknown.

Introduction

Somatostatin is a small peptide, known as a potent inhibitor of growth hormone release (1,2). It has recently been suggested that this neuropeptide is also involved in haematological and granulomatous diseases such as malignant lymphomas, sarcoidosis and human tuberculosis (3,4). Specific receptors for somatostatin have been found in activated lymphocytes, monocytes, and malignant lymphoid cells, by classic ligand binding techniques as well as by in vitro autoradiography (4-8).

The somatostatin analogue [^{111}In-DTPA-D-Phe^{1}]-octreotide has been used successfully for in vivo visualization of a variety of neuroendocrine tumours,

as well as malignant lymphomas and granulomas (3,4,9,10). In one patient with sarcoidosis and arthralgia we observed a high uptake of [^{111}In-DTPA-D-Phe^{1}]-octreotide in the affected joints. This observation led us to investigate the possible use of somatostatin receptor scintigraphy of affected joints in rheumatoid arthritis.

Rheumatoid arthritis is characterized by synovitis. The histopathological features lack uniformity, especially in more established disease, but diffuse lymphocytic infiltration (predominantly CD4 positive lymphocytes) and/or lymphocytic aggregates positioned around postcapillary venules are present in most cases. We postulated that these activated lymphocytes and monocytes in the synovial membranes express somatostatin receptors in sufficient numbers to allow in vivo visualization, as was the case in the disorders mentioned above. Herein we report our first results of somatostatin receptor scintigraphy of the joints in 14 consecutive patients with active rheumatoid arthritis and four selected patients with severe osteoarthritis resembling rheumatoid arthritis. The synovial tissues of two of these patients with rheumatoid arthritis as well as of five other patients were investigated for the presence of specific somatostatin receptors in vitro by using binding assays.

Materials and methods

Patients

Fourteen consecutive patients with rheumatoid arthritis, fulfilling the criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (11), and four selected patients with severe osteoarthritis, all from the Department of Rheumatology at Dr. Daniel den Hoed Clinic, Rotterdam, the Netherlands, were enrolled in the study after giving informed consent. Demographic and clinical characteristics of the patients are shown in Table 1. Clinical evaluation of the joints was performed by a rheumatologist. The joints were scored for pain and swelling on a 0-3 scale, with 0 representing no swelling or pain and 3 representing extensive pain and swelling. Twenty patients with ophthalmic Graves' disease, five with neuroendocrine tumours, and five with malignant lymphomas were used as control patients. None of these patients had signs of arthritis, except for one patient with ophthalmic Graves' disease who had severe generalized joint

pains but no objective abnormalities found on physical examination. The joints of these control patients were scored according to their uptake of radioactivity, similar to the procedure in the patients with rheumatoid arthritis and osteoarthritis.

Somatostatin receptor scintigraphy

The somatostatin analogue [DTPA-D-Phe^1]-octreotide was obtained from Mallinckrodt (Petten, the Netherlands). [DTPA-D-Phe^1]-octreotide was coupled to ^{111}In as described previously (12). [^{111}In-DTPA-D-Phe^1]-octreotide is excreted mainly through the kidneys, 90% of the dose being present in the urine 24 hours after injection. Because of its relatively long effective half-life, [^{111}In-DTPA-D-Phe^1]-octreotide can be used to efficiently visualize somatostatin receptor bearing lymphomas, granulomas, and neuroendocrine tumours after 24 hours when interfering background radioactivity is minimized by renal clearance (13,14). [^{111}In-DTPA-D-Phe^1]-octreotide (200-250 MBq) was injected, and planar images were obtained with a large-field-of-view gamma camera (Counterbalance 3700 and ROTA II; Siemens Gammasonics, Erlangen, Germany) equipped with a medium energy collimator. Scanning was done for a preset time of 15 minutes, 24 hours after injection of the ^{111}In coupled somatostatin analogue (14). In normal individuals, physiologic accumulation of radioactivity is seen 24 hours after administration of [^{111}In-DTPA-D-Phe^1]-octreotide in the pituitary and thyroid glands, the liver, the spleen, and the kidneys, as well as the urinary bladder. Radioactivity at other sites is considered to be abnormal and indicates the presence of abnormal somatostatin receptor positive tissue. To define the localizations of somatostatin receptors as visualized during this scanning procedure, the joints were scored on a 0-3 scale, where 0 = no uptake, 1 = low uptake, 2 = moderate uptake, and 3 = high uptake. The scintigrams of all patients were analyzed by two examiners who were not aware of the patient's identity, medical history, or outcomes of other investigations. In order to compare the number of lesions found by physical examination with those visualized during somastostatin receptor scintigraphy, the images were divided into easily recognizable parts. At physical examination a total of 42 joints per patient had been evaluated: the shoulders, elbows, wrists, metacarpophalangeal joints, distal interphalangeal joints, hips, knees, ankles, and metatarsophalangeal joints. All these joints were also evaluated and scored on the scintigrams.

Somatostatin receptor autoradiography

The presence of somatostatin receptors in synovial tissue was investigated by autoradiography on 10 μm thick frozen sections using an iodinated somatostatin octapeptide analogue, [^{125}I-Tyr^3]-octreotide. Tissue sections were mounted on precleaned microscope slides and stored at -20°C for at least 3 days to improve adhesion of tissue to the slide. Sections were then incubated for 2 hours at room temperature in the presence of the iodinated ligand (0.15-0.30 x 10^6 dpm/ml, about 80-160 pM). The incubation solution was 170 mM Tris-HCl buffer, pH 7.4, containing 1% bovine serum albumin (BSA), bacitracin (40 μg/ml) and $MgCl_2$ (5 mM). Non-specific binding was determined by adding 1 μM solution of unlabelled octreotide. Incubated sections were washed twice for 5 minutes in cold incubation buffer containing 0.25% BSA and then in buffer alone, and dried quickly. The sections were exposed to ^{3}H labelled ultrafilms (Cambridge Research, Nussdorf, Germany) and maintained for one week in X-ray cassettes. Displacement experiments were done on successive sections of synovial membranes. In addition, saturation experiments using increasing concentrations of octreotide were performed on tissue sections incubated with radioligand (30,000 cpm/100 μl). The autoradiograms were quantified using a computer assisted image processing system. Tissue standards for iodinated compounds (Amersham, Aylesburg, United Kingdom) were used for this purpose. Synovial tissue was designated somatostatin receptor positive when the optical density in the 'total binding' section was at least twice the optical density of the 'non-specific' binding section. These techniques have been described previously in detail for various types of tumorous and non-tumorous tissues (15-18). Autoradiography was performed on synovial biopsy specimen from two patients who underwent scintigraphy and five other patients who did not undergo scintigraphy, from whom synovial membrane tissue was obtained in the course of a surgical procedure.

Results

The results of somatostatin receptor scintigraphy in the 14 patients with rheumatoid arthritis and the four patients with osteoarthritis are listed in Table 1. We observed uptake of [^{111}In-DTPA-D-Phe^1]-octreotide in a number of joints of all of the patients with rheumatoid arthritis (Figures 1-4).

Table 1. Patient characteristics.

Patient	Sex*	Age	Rheumatoid factor	Erythrocyte sedimentation rate (mm/hour)	Treatment	Number of joints		
						Swollen	Painful	Somatostatin receptor positive
Rheumatoid arthritis								
1	F	72	+	59	Corticosteroids, chloroquine, NSAID†	17	12	24
2	M	73	+	28	Corticosteroids, chloroquine, NSAID†	7	15	14
3	F	67	+	69	Corticosteroids, chloroquine, methotrexaat, NSAID†	34	23	34
4	F	38	-	30	Gold	34	34	27
5	F	74	-	-	Corticosteroids, sulfasalazine	10	16	14
6	F	66	+	39	NSAID†	21	21	8
7	F	55	+	79	NSAID†	6	6	8
8	F	80	+	66	NSAID†	24	38	35
9	M	52	+	56	Azathioprine, NSAID†	14	19	31
10	M	77	+	94	NSAID†	14	0	22

Continued...

Table 1. (cont.)

Patient	Sex*	Age	Rheumatoid factor	Erythrocyte sedimentation rate (mm/hour)	Treatment	Number of joints		
						Swollen	Painful	Somatostatin receptor positive
Rheumatoid arthritis (cont.)								
11	F	65	+	58	Corticosteroids, NSAID†	10	14	33
12	F	59	-	48	D-penicillamine, corticosteroids, NSAID†	20	29	36
13	M	68	+	87	NSAID†	30	28	20
14	F	32	+	80	Gold, NSAID†	33	35	40
Osteoarthritis								
15	F	68	-	24	NSAID†	12	1	6
16	F	84	-	18	Acetaminophen	1	7	6
17	F	65	-	12	None	12	17	6
18	F	77	-	10	NSAID†	4	5	8

* M = male; F = female

† NSAID = non-steroidal antiinflammatory drug

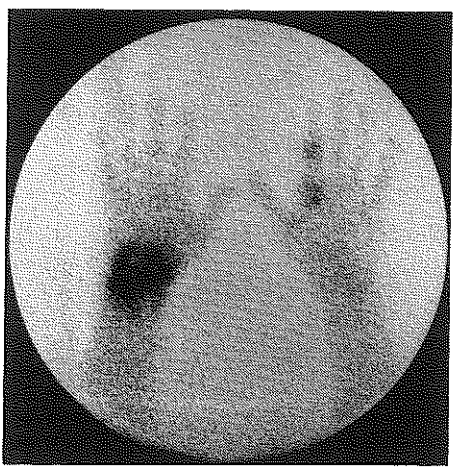

Figure 1. Visualization of the affected right wrist and left second metacarpophalangeal and proximal interphalangeal joints of patient 8 (see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

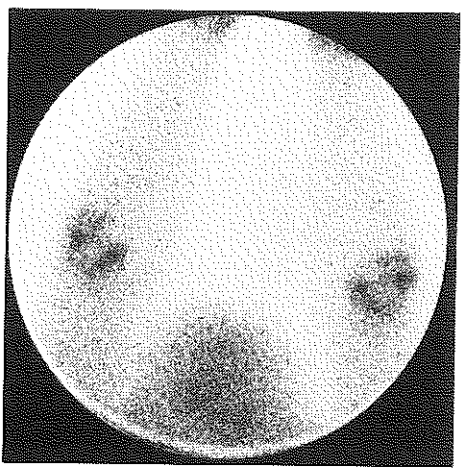

Figure 2. ^{111}In labelled somatostatin analogue scintigraphy, visualizing both affected elbows of patient 8 (see Table 1) (24 hours after injection).

Table 2 illustrates the relationships between somatostatin receptor scintigraphy findings and pain and swelling in the patients with rheumatoid arthritis. Five hundred and seventy-six joints were scored on the scintigrams. In two patients a total of 12 joints (all metatarsophalangeal joints in patient 1 and both shoulders in patient 11) could not be scored due to technical reasons. Two hundred and twenty (76%) of the 290 painful joints in the 14 patients with rheumatoid arthritis were somatostatin receptor positive, whereas 125 (44%) of the remaining 286 non-painful joints were also positive. Of the remaining 70 painful joints that were negative on the somatostatin receptor scan, 32 were in two patients (patient 6 and 11). They did not differ from the other 12 patients with regard to either treatment or clinical symptoms. The median pain score was higher in the patients whose joints were positive at scintigraphy ($p<0.0001$, Wilcoxon rank-sum test). The sensitivity of scintigraphy for swollen joints was also 76% (207 of 274). Thirty-six of the 67 swollen joints that were negative at scintigraphy were also found in patients 6 and 11. Of 302 joints that were not swollen, 138 (46%) were somatostatin receptor positive. The median degree of swelling

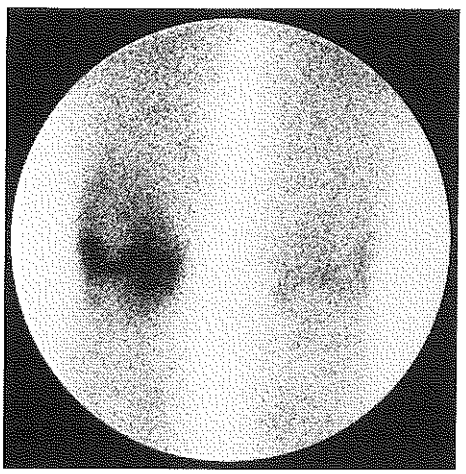

Figure 3. Visualization of the moderately painful and swollen right knee of patient 1 (see Table 1) by ^{111}In labelled somatostatin analogue scintigraphy (anterior view, 24 hours after injection).

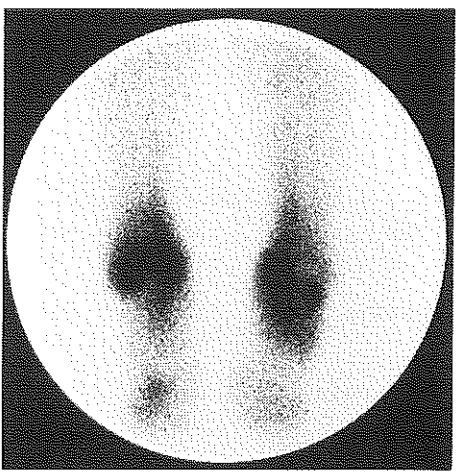

Figure 4. ^{111}In labelled somatostatin analogue scintigraphy, visualizing the affected ankles and right forefoot of patient 7 (see Table 1) (anterior view, 24 hours after injection).

correlated well with positivity at scintigraphy ($p<0.0001$, Wilcoxon rank-sum test). The sensitivity of scintigraphy for painful and/or swollen joints was 74% (252 of 340). Ninety-three of 236 joints without clinical involvement (39%) were visualized. When the combination 'pain and swelling' was scored, 80% of the joints (175 of 220) were positive at scintigraphy. In patient 13 uptake of radioactivity was seen in the pericardium and pleurae, which was related to extraarticular rheumatoid arthritis involvement.

In the patients with osteoarthritis, the sensitivity of scintigraphy for joint swelling and joint pain was only 20% and 14%, respectively, and the frequency of positive findings did not differ significantly between affected and clinically unaffected joints ($p>0.05$, Chi-square test). The median uptake of radioactivity in the patients with osteoarthritis was lower than that in patients with rheumatoid arthritis (85% of the osteoarthritis group had a scintigraphy score of 1, versus 36% of the rheumatoid arthritis group). In none of the control patients with ophthalmic Graves' disease, neuroendocrine tumours, or malignant lymphomas uptake of radioactivity was observed in the joints.

Table 2. Relationship between joint pain/swelling and somatostatin receptor scintigraphy results in 14 patients with rheumatoid arthritis.

		Scintigraphy		
		Number of negative joints (%)	Number of positive joints (%)	Total number of joints
Pain	No	161 (56%)	125 (44%)	286
	Yes	70 (24%)	220 (76%)	290
Swelling	No	164 (54%)	138 (46%)	302
	Yes	67 (24%)	207 (76%)	274
Pain and/or swelling	No	143 (61%)	93 (39%)	236
	Yes	88 (26%)	252 (74%)	340
Pain and swelling	No	186 (52%)	170 (48%)	356
	Yes	45 (20%)	175 (80%)	220

The presence of specific somatostatin receptors in surgically excised synovial tissues was confirmed by in vitro autoradiography in two patients with rheumatoid arthritis. Both joints had also been visualized in vivo by scintigraphy. The displacement curve shown in Figure 5 illustrates the high affinity and specificity of the [^{125}I-Tyr^{3}]-octreotide binding in studies with these synovial tissue sections. This suggests the presence of somatostatin receptors in the tissue. However, we were unable to identify by autoradiographic studies which cell type(s) expressed somatostatin receptors in this tissue. The presence of specific somatostatin receptors was confirmed in four of five other patients with active rheumatoid arthritis who did not undergo in vivo scintigraphy, from whom metacarpophalangeal, knee, and ankle synovial membrane tissue samples were obtained.

Discussion

Somatostatin receptor scintigraphy has been used successfully in the visualization of neuroendocrine tumours as well as granulomatous diseases and malignant lymphomas (3,4,9,10). This is, to our knowledge, the first report of the use of this technique in the visualization of clinically affected joints in rheumatoid arthritis. Rheumatoid arthritis is a chronic inflammatory

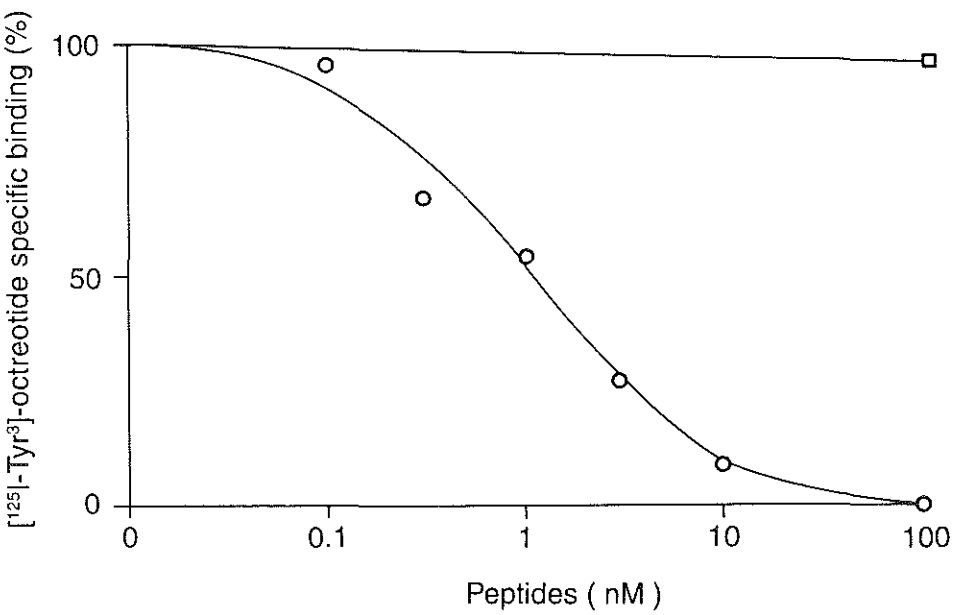

Figure 5. In vitro detection of somatostatin receptors in a synovial tissue sample from a rheumatoid arthritis patient. The displacement curve of [^{125}I-Tyr^3]-octreotide is shown in tissue sections incubated with the radioligand (30,000 cpm/100 µl) and increasing concentrations of unlabelled octreotide (○) or 100 µM of epidermal growth factor (□).

disorder involving the synovial membranes of multiple joints. There is no 'gold standard' for the assessment of the clinical activity of the disease. Subjective parameters are mainly used to quantify arthritis activity (19). Pain and swelling of the joints are the main clinical indicators of inflammatory activity in rheumatoid arthritis. In the 14 patients with active rheumatoid arthritis examined in this study, clinical activity correlated well with the results of somatostatin receptor scintigraphy. However, in two patients, a number of painful and swollen joints were negative at scintigraphy. The reason for this discrepancy in these two patients is not clear. The number of false positive scintigrams is unknown, because we did not perform synovial biopsies to exclude subclinical synovitis in these patients. Pleural and pericardial involvement of rheumatoid arthritis were visualized as well in one patient, suggesting that somatostatin receptor scintigraphy is also useful in the visualization of extraarticular involvement.

In the selected patients with severe osteoarthritis, no significant correlation between pain and/or swelling and uptake of radioactivity by the affected joints at scintigraphy was demonstrated. However, the osteoarthritic joints demonstrated a lower uptake of [111In-DTPA-D-Phe ^{1}]-octreotide in comparison with the joints of the patients with rheumatoid arthritis. We cannot rule out the presence of an inflammatory component in the affected joints of these selected osteoarthritis patients; this is substantiated by the finding of negative scan results in all of the 'control' patients.

Different scintigraphic techniques have been used over the years for the visualization of affected joints in rheumatoid arthritis (20-25). The markers used can be classified according to the compartment involved. The vascular compartment reflects the increased blood flow and interstitial extravasation, the bone compartment reflects the normal response to bone destruction, and the inflammatory compartment reflects the infiltration of leucocytes. To our knowledge, no scintigraphic technique related to specific receptors in the affected synovial membranes in rheumatoid arthritis has been described previously. In vitro binding assays showed the presence of specific somatostatin receptors in synovial biopsy specimen from our patients, but did not enable the recognition of the type of cells that expressed these somatostatin receptors. [^{111}In-DTPA-D-Phe^{1}]-octreotide may bind to somatostatin receptors on lymphocytes and monocytes that have infiltrated into the affected synovial membranes. The low grade uptake of radioactivity in osteoarthritis, which has only a minor inflammatory component, supports this hypothesis.

The specificity of this in vivo method of somatostatin receptor visualization might be substantiated by investigation of the effect of simultaneous administration of high dose 'cold' octreotide. However, our in vitro studies already demonstrated specific binding of octreotide, which could be completely prevented by the addition of excess 'cold' octreotide, in the synovial membranes studied.

The relationship between somatostatin receptor expression and disorders of the immune system have been described previously (3-8,26-35). T and B lymphocytes and monocytes express somatostatin receptors, and somatostatin has been shown to modulate the activity of the immune system (5-7,26,27,31). At low concentrations, somatostatin caused inhibition of the

lectin induced proliferation response of lymphocytes (26,27,31). Inhibition of cytotoxicity and of the release of cytokines by lymphocytes has also been described (33,35). Somatostatin suppresses immunoglobulin synthesis in Peyer's patches as well as in a myeloma cell line (28,30). Lymphoid cell lines of T cell origin have been shown to express somatostatin receptors (29). Somatostatin and somatostatin receptors have also been detected in schistosomiasis induced murine granulomas (32,34). Treatment with the long acting somatostatin analogue octreotide decreased the granulomatous response in this model by approximately 45% (36). In ten patients with active rheumatoid arthritis, a transient beneficial effect was observed after direct intraarticular administration of somatostatin (37). This may be related to the inhibition of the release of substance P by nerve endings within these joints or by modulation of the activity of infiltrating immune cells. Thus, the potential value of somatostatin in the treatment of rheumatoid arthritis, as well as in the monitoring of disease activity, merits further exploration.

References

1. Reichlin S. Somatostatin (first of two parts). N Engl J Med 1983;309:1495-501.
2. Reichlin S. Somatostatin (second of two parts). N Engl J Med 1983;309:1556-63.
3. van Hagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY, Löwenberg B, Lamberts SWJ. Somatostatin analogue scintigraphy of malignant lymphomas. Br J Haematol 1993;83:75-9.
4. van Hagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue JA, Hoogsteden HC, Lamberts SWJ. Somatostatin analogue scintigraphy in granulomatous diseases. Eur J Nucl Med 1994;21:497-502.
5. Sreedharan SP, Kodama KT, Peterson KE, Goetzl EJ. Distinct subsets of somatostatin receptors on cultured human lymphocytes. J Biol Chem 1989;264:949-52.
6. Hiruma K, Koike T, Nakamura H, Sumida T, Maeda T, Tomioka H, Yoshida S, Fujita T. Somatostatin receptors on human lymphocytes and leukaemia cells. Immunology 1990;71:480-5.
7. Reubi JC, Horisberger U, Waser B, Gebbers JO, Laissue JA. Preferential location of somatostatin receptors in germinal centers of human gut lymphoid tissue. Gastroenterology 1992;103:1207-14.
8. Reubi JC, Waser B, van Hagen PM, Lamberts SWJ, Krenning EP, Gebbers JO, Laissue JA. In vitro and in vivo detection of somatostatin receptors in human malignant lymphomas. Int J Cancer 1992;50:895-900.
9. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.
10. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. [Review] Endocr Rev 1991;12:450-82.

11. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
12. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, Lamberts SWJ, Visser TJ, Krenning EP. [^{111}In-DTPA-D-Phe^{1}]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991;49:1583-91.
13. Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyono-Han B, de Jong M, Kooij PPM, Bruns C, van Hagen PM, Marbach P, Visser TJ, Lamberts SWJ. In vivo application of [^{111}In-DTPA-D-Phe ^{1}]-octreotide for detection of somatostatin receptor-positive tumors in rats. Life Sci 1991;49:1593-601.
14. Krenning EP, Bakker WH, Kooij PPM, Breeman WAP, Oei HY, de Jong M, Reubi JC, Visser TJ, Kwekkeboom DJ, Reijs AEM, van Hagen PM, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^{1}]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^{3}]-octreotide. J Nucl Med 1992;33:652-8.
15. Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
16. Reubi JC, Maurer R. Autoradiographic mapping of somatostatin receptors in the rat central nervous system and pituitary. Neuroscience 1985;15:1183-93.
17. Reubi JC, Maurer R, Klijn JGM, Stefanko SZ, Foekens JA, Blaauw G, Blankenstein MA, Lamberts SWJ. High incidence of somatostatin receptors in human meningiomas: biochemical characterization. J Clin Endocrinol Metab 1986;63:433-8.
18. Reubi JC, Maurer R, von Werder K, Torhorst J, Klijn JGM, Lamberts SWJ. Somatostatin receptors in human endocrine tumors. Cancer Res 1987;47:551-8.
19. Bull BS, Westengard JC, Farr M, Bacon PA, Meyer PJ, Stuart J. Efficacy of tests used to monitor rheumatoid arthritis. Lancet 1989;2:965-7.
20. Ahlstrom S, Gedda PO, Hedberg H. Disappearence of radioactive serum albumin from joints in rheumatoid arthritis. Acta Rheumatol Scand 1956;2:129-36.
21. McCall IW, Sheppard H, Haddaway M, Park WM, Ward DJ. ^{67}Gallium scanning in rheumatoid arthritis. Br J Radiol 1983;56:241-3.
22. Uno K, Matsui N, Nohira K, Suguro T, KitakataY, Uchiyama G, Miyoshi T, Uematsu S, Inoue S, Arimizu N. ^{111}Indium leukocyte imaging in patients with rheumatoid arthritis. J Nucl Med 1986;27:339-44.
23. O'Sullivan MM, Powell N, French AP, Williams KE, Morgan JR, Williams BD. Inflammatory joint disease: a comparison of liposome scanning, bone scanning, and radiography. Ann Rheum Dis 1988;47:485-91.
24. Rosenthal LL. Nuclear medicine techniques in arthritis. [Review] Rheum Dis Clin North Am 1991;17:585-97.
25. Rydgren L, Wollmer P, Hultquist R, Gustavson T. ^{111}Indium-labelled leukocytes for measurement of inflammatory activity in arthritis. Scand J Rheumatol 1991;20:319-25.
26. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T-lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.

27. Pawlikowski M, Stepien H, Kunert-Radek J, Schally AV. Effect of somatostatin on the proliferation of mouse spleen lymphocytes in vitro. Biochem Biophys Res Commun 1985;129:52-5.
28. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
29. Nakamura H, Koike T, Hiruma K, Sato T, Tomioka H, Yoshida S. Identification of lymphoid cell lines bearing receptors for somatostatin. Immunology 1987;62:655-8.
30. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. J Immunol 1988;141:937-41.
31. Malec P, Zeman K, Markiewicz K, Tchorzewski H, Nowak Z, Baj Z. Short-term somatostatin infusion affects T lymphocyte responsiveness in humans. Immunopharmocology 1989;17:45-9.
32. Weinstock JV, Blum AM, Malloy T. Macrophages within the granulomas of murine *Schistosoma mansoni* are a source of a somatostatin 1-14-like molecule. Cell Immunol 1990;131:381-90.
33. Yousefi S, Vaziri N, Carandang G, Le W, Yamamoto R, Granger G, Ocariz J, Cesario T. The paradoxical effects of somatostatin on the bioactivity and production of cytotoxins derived from human peripheral blood mononuclear cells. Br J Cancer 1991;64:243-6.
34. Weinstock JV. Production of neuropeptides by inflammatory cells within the granulomas of murine schistosomiasis mansoni. [Review] Eur J Clin Invest 1991;21:145-53.
35. Agro A, Padol I, Stanisz AM. Immunomodulatory activities of the somatostatin analogue BIM 23014c: effects on murine lymphocyte proliferation and natural killer activity. Regul Pept 1991;32:129-39.
36. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. [Review] Clin Immunol Immunopathol 1992;64:17-22.
37. Matucci-Cerinic M, Marabini S. Somatostatin treatment for pain in rheumatoid arthritis: a double blind versus placebo study in knee involvement. Med Sci Res 1988;16:233-4.

CHAPTER 5.2

VASCULAR SOMATOSTATIN RECEPTORS IN RHEUMATOID ARTHRITIS

Abstract

The peripheral nervous system and its neuropeptidergic pathways may play an important role in the pathogenesis and development of rheumatoid arthritis. In the present study, the role of the neuropeptide somatostatin, which was recently shown to be implicated in inflammatory diseases of the gastrointestinal tract, was evaluated by measuring the expression of somatostatin receptors in synovium from patients with rheumatoid arthritis. Somatostatin receptors were detected using in vitro receptor autoradiography in the synovium from five patients with active disease. No receptors were found in one case, a successfully treated patient with quiescent disease. The receptors were of high affinity and specific for biologically active somatostatin analogues. Displacement by nanomolar concentrations of somatostatin 14, somatostatin 28, and octreotide was observed, suggesting that most of the receptors identified belong to the somatostatin receptor subtype 2. The somatostatin receptors were preferentially located in blood vessels, with specific labelling of the veins but not of the arteries. The whole vessel wall was homogeneously labelled, including the smooth muscle cells and probably the endothelium. These data suggest that the synovium in active rheumatoid arthritis expresses a high density of somatostatin receptors. Somatostatin may act through these venous receptors to influence the inflammatory process by induction of vasoconstriction, inhibition of plasma extravasation and cell migration, or inhibition of neovascularization.

Introduction

Clinical and experimental observations suggest the implication of the nervous system in the etiology and pathogenesis of human joint diseases (1-3): for instance, neurological lesions or stress are known to affect the development and severity of rheumatoid arthritis lesions (4-6). The human nervous system is also able to modulate inflammatory processes in peripheral tissues, in particular via blood vessels (7,8). There is good evidence that both the afferent (sensory) and efferent (sympathetic) components of the peripheral nervous system contribute to the inflammatory process (1).

Neuropeptides play a major role within the peripheral nervous system (9). Moreover, several neuropeptides and their receptors, in particular substance P, calcitonin gene related peptide and endothelin are known to be involved in joint

physiology, and in joint diseases, such as rheumatoid arthritis (10,11). There is, for instance, experimental evidence that substance P can be released from peripheral nerve terminals into the joints (12) and that substance P nerve fibres are involved in the local regulation of blood flow, vascular permeability and smooth muscle tone (13,14). In rheumatoid arthritis, the concentration of substance P is elevated in the synovial fluid (13,15,16), and substance P receptors have been identified in synovial blood vessels (17). Similarly, in synovial tissue from patients with rheumatoid arthritis, endothelial cells in blood vessels display endothelin-like immunoreactivity and endothelin binding sites (18). Therefore, substance P and endothelin may influence the course of joint disease.

Somatostatin, a small neuropeptide, has a wide range of actions within the central and peripheral nervous system, and the immune system (19). These actions are mediated by specific somatostatin receptors located in all these target tissues (20-22). Somatostatin and somatostatin receptors are also implicated in many pathological conditions. For instance, a great variety of human neoplasms express somatostatin receptors in high density (23,24). Recently, somatostatin was shown to be involved in inflammatory diseases: active granulomatous lesions in sarcoidosis and human tuberculosis express a high density of somatostatin receptors (25). Moreover, in Crohn's disease and ulcerative colitis, the intestines express somatostatin receptors on blood vessels in the inflamed areas (26). These data suggest an important role of somatostatin in these pathological conditions, and have led to useful therapeutic and diagnostic applications of somatostatin analogues as well (23).

The aim of the present study was to investigate whether or not somatostatin receptors are expressed in rheumatoid arthritis. Somatostatin receptor autoradiography (20,27) was used to localize and characterize somatostatin receptors in biopsies of synovial tissue from rheumatoid arthritis patients, using either [^{125}I-Tyr^{3}]-octreotide (28) or the [^{125}I-Leu^{8}-D-Trp^{22}-Tyr^{25}]-somatostatin 28 (29) as radioligands.

Materials and methods

Patients

Samples of synovial tissue were obtained during replacement surgery in six

patients with rheumatoid arthritis. All samples were frozen without delay after surgical resection and stored at -70°C for 4-12 months. The diagnosis of rheumatoid arthritis was made according to standard clinical, radiological and histological criteria (30). The patient characteristics, the sites of surgical intervention and the histological findings in the samples are summarized in Table 1.

Somatostatin receptor autoradiography

Cryostat sections of the tissue samples, 10-20 μm thick, were processed for somatostatin receptor autoradiography as described in detail previously (20,27). The radioligands used were the somatostatin analogues [^{125}I-Tyr^{3}]-octreotide and [^{125}I-Leu^{8}-D-Trp^{22}-Tyr^{25}]-somatostatin 28, known to specifically label somatostatin receptors. Both ligands were iodinated, purified on high pressure liquid chromatography columns (specific activity 2000 Ci/mmol) and characterized in standard binding assays as described previously (28,29). For autoradiography, tissue sections were mounted on microscope slides and stored at -20°C for at least 3 days to improve adhesion of tissue to the slide. Sections were then incubated for 2 hours at ambient temperature in the presence of the iodinated ligand (0.15-0.30 x 10^{6} dpm/ml, approximately 40-80 pM). The incubation solution was Tris-HCl buffer (170 mM, pH 8.2), containing 1% bovine serum albumin (BSA), bacitracin (40 μg/ml), and $MgCl_2$ (5 mM) to inhibit endogenous proteases. Non-specific binding was determined by adding 1 μM unlabelled [Tyr^{3}]-octreotide or somatostatin 28. Incubated sections were washed twice for 5 minutes in cold incubation buffer containing 0.25% BSA, then in buffer alone, and dried quickly. Finally, the sections were apposed to ^{3}H labelled ultrafilms (Amersham, United Kingdom) and exposed for one week in X-ray cassettes. In selected cases, displacement experiments were performed with successive tissue sections by means of increasing concentrations of various biologically active (somatostatin 14, somatostatin 28, octreotide, [Tyr^{3}]-octreotide) or inactive somatostatin analogues (somatostatin 28-(1-12)) and an unrelated peptide (epidermal growth factor). The autoradiograms were quantified using a previously described computer assisted image processing system (27). Tissue standards for iodinated compounds (Amersham, United Kingdom) were used for this purpose. A tissue was defined as somatostatin receptor positive when the optical density measured over an area in the total binding section was at least twice the optical density of the non-specific binding section (27). Adjacent sections from selected samples of tissues tested with [^{125}I-Tyr^{3}]-octreotide were also examined for specific binding with the radioligand [^{125}I-Leu^{8}-D-Trp^{22}-Tyr^{25}]-

somatostatin 28, to confirm that the same tissue elements were identified by both ligands. Adjacent sections were also studied by conventional histological techniques and immunohistochemical staining for better visualization and identification of blood vessels (smooth muscle actin, factor VIII), to establish the structural identity of somatostatin receptor positive tissues.

Results

All synovial tissue biopsies with histological signs of active rheumatoid arthritis showed specific binding of the radioligands [^{125}I-Tyr^{3}]-octreotide and [^{125}I-Leu^{8}-D-Trp^{22}-Tyr^{25}]-somatostatin 28 (Table 1). The distribution was patchy in all specimens, suggesting that only selected structures were able to bind the somatostatin radioligands. Figure 1 shows an example at low magnification of the distribution of somatostatin receptors labelled by [^{125}I-Tyr^{3}]-octreotide in a synovial biopsy. The patchy distribution of the radioactivity correlates well with the distribution pattern of smooth muscle actin immunoreactivity. The somatostatin receptors were of high affinity and specific for somatostatin. As seen in Figure 2, bound [^{125}I-Tyr^{3}]-octreotide could be displaced in the nanomolar range by somatostatin 28 or octreotide, but not by the biologically inactive somatostatin 28-(1-12), nor by epidermal growth factor. A biopsy from patient 6, with biochemically and clinically proven rheumatoid arthritis, showed no histological signs of active rheumatoid arthritis after successful treatment with cytostatic drugs. The synovial tissue specimen displayed a low density of vessels on which no somatostatin receptors could be identified (Table 1).

The cellular localization of the somatostatin receptors was investigated by comparing the somatostatin receptor autoradiography results with the corresponding tissue section stained for special structures. As can be seen in Figure 3A-3C, the somatostatin receptors were clearly localized on blood vessels. Indeed, on comparison of the pattern of somatostatin receptors with the corresponding section stained for smooth muscle actin, it became evident that veins expressed somatostatin receptors, whereas arteries did not. A similar picture was seen in another case, shown in Figure 3D-3F, where the pattern of somatostatin receptors was found to correspond with the localization of veins as depicted by immunohistochemical staining for factor VIII. Here again, the veins were somatostatin receptor positive whereas the arteries were not labelled; in all cases, the entire venous wall was receptor positive. Most of the

Table 1. Patient characteristics and somatostatin receptor status in six patients with rheumatoid arthritis.

Patient	Sex*	Age	Diagnosis	Therapy	Site	Histology	Somatostatin receptors	
							Autoradiography	Scintigraphy
1	F	59	Rheumatoid arthritis	Salazopyrine Indomethacin	Toes	Rheumatoid arthritis compatible synovitis†; leucocyte infiltration of some blood vessels	Positive	Not done
2	F	64	Rheumatoid arthritis	Prednisone	Wrist	Rheumatoid arthritis compatible synovitis†	Positive	Not done
3	F	75	Rheumatoid arthritis	Prednisone Salazopyrine	Knee	Rheumatoid arthritis compatible synovitis†	Positive	Positive
4	F	64	Rheumatoid arthritis	Voltaren	Wrist	Rheumatoid arthritis compatible synovitis†	Positive	Not done
5	M	67	Rheumatoid arthritis T cell lymphoma	None	Fingers	Rheumatoid arthritis compatible synovitis†	Positive	Positive
6	F	61	Rheumatoid arthritis‡	Methotrexate	Fingers	Synovial tissue without significant inflammatory changes	Negative	Not done

* M = male; F = female

† Lymphoplasmocytic infiltrate, fibrinous exudate, occasional lymphatic follicles, occasional granulomas with central fibrinoid necrosis; no vasculitis.

‡ Rheumatoid arthritis was diagnosed several years before joint replacement, and the patient had been succesfully treated with methotrexate; at the time of joint replacement, quiescent arthritis was diagnosed.

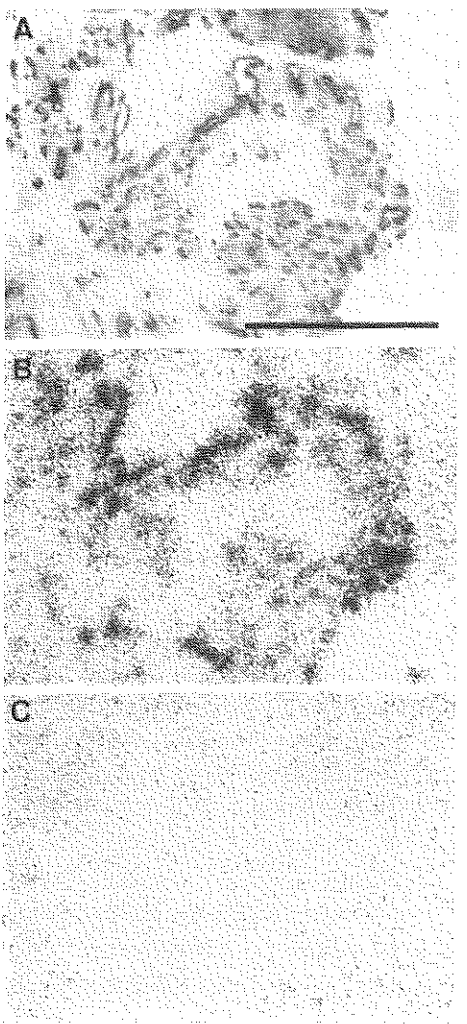

Figure 1. Visualization of somatostatin receptors in a synovial membrane from a patient with rheumatoid arthritis. (A) Immunohistochemical staining for smooth muscle actin, with intensively labelled blood vessels. (B) Autoradiogram showing total binding of [^{125}I-Tyr^{3}]-octreotide. A non-homogeneous, patchy distribution of the label is seen, related to the vessel distribution. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^{3}]-octreotide in the presence of 1 μM octreotide. Bar represents 1 mm.

synovial tissues showed inflammatory signs such as lymphoplasmocytic infiltrates, but the wall of the veins did not consistently display inflammatory changes. A receptor positive vein in a rheumatoid arthritis sample without inflammatory infiltrate is seen in Figure 4. This suggests that the somatostatin receptors are likely to be located on the smooth muscle cells, and perhaps also on the endothelial cells of these veins, rather than on occasional infiltrating leucocytes. In general, the veins were the most prominently labelled structures

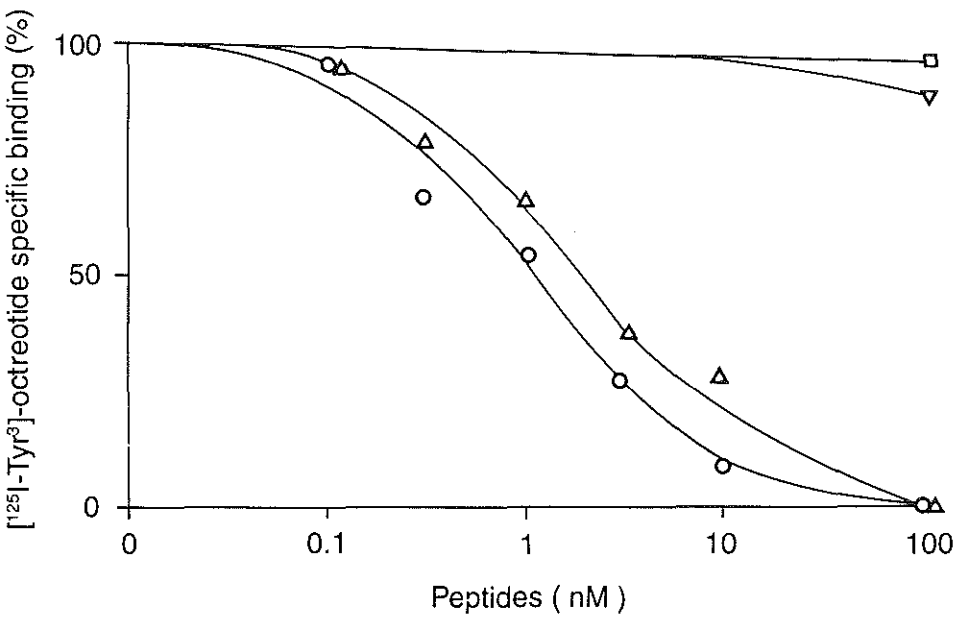

Figure 2. Displacement curve of [^{125}I-Tyr^{3}]-octreotide in tissue sections from a synovial membrane of a patient with rheumatoid arthritis. Tissue sections were incubated with [^{125}I-Tyr^{3}]-octreotide (30,000 cpm/100 µl), and increasing concentrations of unlabelled octreotide (○) or somatostatin 28 (△), or 100 nM of somatostatin 28-(1-12) (▽) or epidermal growth factor (□). Each point represents the optical density of binding measured in a region rich in blood vessels in at least two sections. Non-specific binding, in the presence of 100 nM octreotide, was subtracted from all values.

in the synovial biopsies, with very little label, if any, on other structures such as normal synovial membranes, connective tissue or lymphocytic infiltrates. However, if lymphatic follicles with germinal centres were present, the germinal centres were labelled as well (21).

Two of the receptor positive patients were investigated in vivo with ^{111}In labelled somatostatin analogue scintigraphy (31,32) before surgical resection of the damaged joints; the joints which could be clearly localized in vivo were shown to have somatostatin receptor positive lesions in vitro.

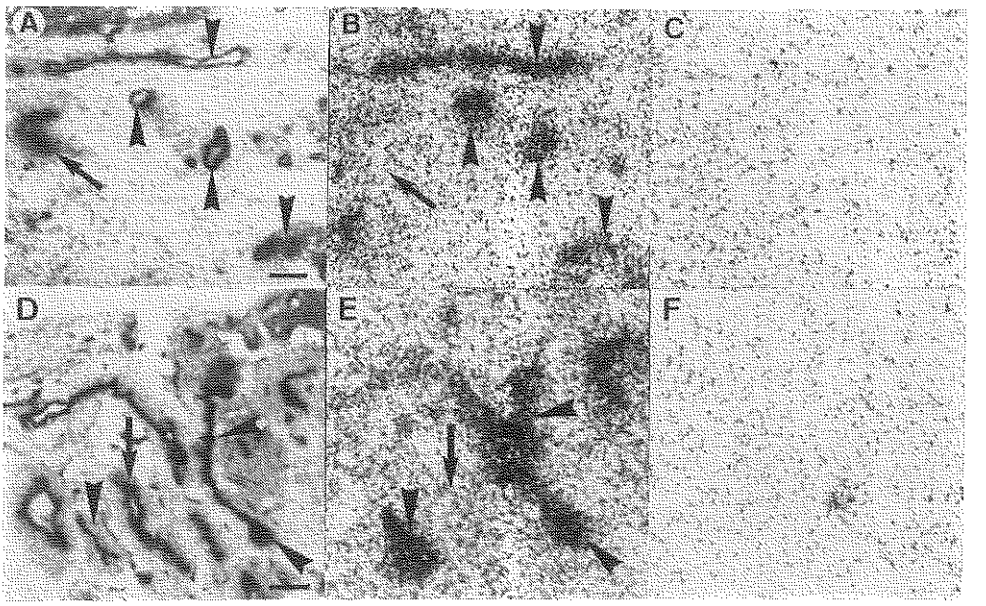

Figure 3. Visualization of somatostatin receptors in blood vessels in synovial membranes from two patients with rheumatoid arthritis. (A) Section stained for smooth muscle actin, showing various veins (arrowheads) and arteries (arrow) in inflamed synovial tissue. (D) Section stained for factor VIII, showing various veins (arrowheads) and arteries (arrow) in inflamed synovial tissue. (B) and (E) Autoradiograms showing total binding of [^{125}I-Tyr^{3}]-octreotide. The veins (arrowheads) are labelled but the arteries (arrows) are not. (C) and (F) Autoradiograms showing non-specific binding of [^{125}I-Tyr^{3}]-octreotide, in the presence of 1 μM octreotide. Bars represent 0.1 mm.

Discussion

This study reports the presence of somatostatin receptors in blood vessels localized in diseased synovial tissues of patients suffering from rheumatoid arthritis. The receptors are homogeneously distributed in the whole wall of veins but are not identified in arteries. The fact that the venous wall rarely contained non-vascular constituents such as infiltrating leucocytes, indicates that the muscular wall and perhaps the endothelium are the most likely sites of somatostatin receptor expression in rheumatoid arthritis.

Somatostatin receptors were identified in all synovial tissues displaying histological changes compatible with active rheumatoid arthritis. Conversely, the synovial biopsy from a patient with clinically inactive rheumatoid arthritis and histological findings compatible with a quiescent stage of the disease does not

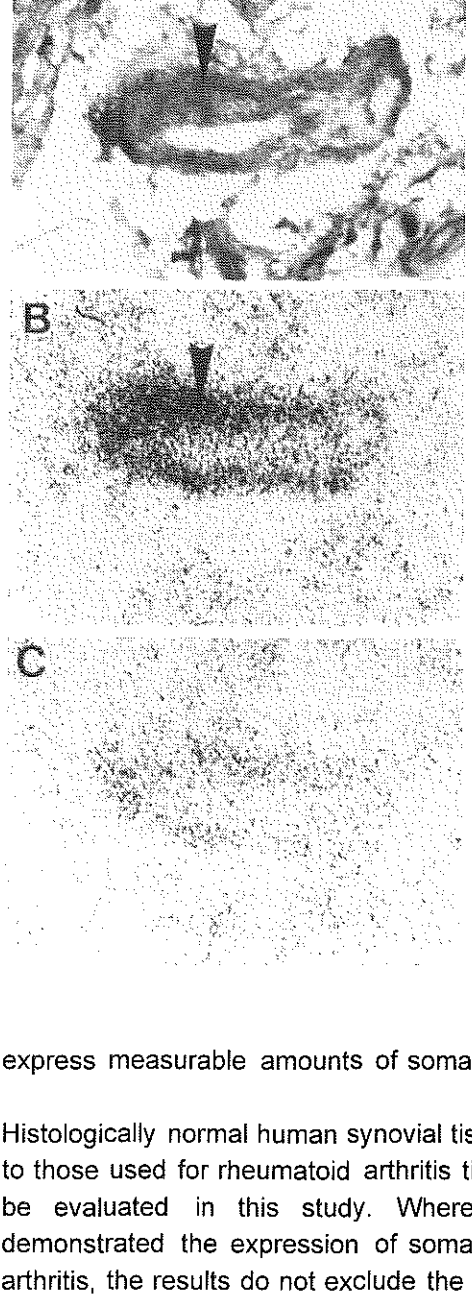

Figure 4. Visualization of somatostatin receptors in a vein in synovial membrane from a patient with rheumatoid arthritis. (A) Haematoxylin-eosin stained section. (B) Autoradiogram showing total binding of [^{125}I-Tyr^{3}]-octreotide. Somatostatin receptors are localized in the entire wall of the vein. (C) Autoradiogram showing non-specific binding of [^{125}I-Tyr^{3}]-octreotide in the presence of 1 μM octreotide. Bar represents 0.1 mm.

express measurable amounts of somatostatin receptors.

Histologically normal human synovial tissue, collected under conditions identical to those used for rheumatoid arthritis tissue, is difficult to obtain and could not be evaluated in this study. Whereas the present investigation clearly demonstrated the expression of somatostatin receptors in active rheumatoid arthritis, the results do not exclude the possibility that normal synovial tissue or

diseased, non-rheumatoid synovia also express somatostatin receptors.

The somatostatin receptors identified in active rheumatoid arthritis tissue have the same characteristics as somatostatin receptors in established somatostatin target tissue, i.e. they are of high affinity and specific for biologically active somatostatin analogues (21-22,33). The displacement of the radioligand by somatostatin 28 as well as by octreotide in the nanomolar range, strongly suggests that the venous somatostatin receptors belong mainly to the somatostatin receptor subtype 2 (34). Indeed, human somatostatin subtype 2 receptors can be distinguished from the other four human somatostatin receptor subtypes by their very high affinity for octreotide (34-36).

The presence of somatostatin receptors on veins and venules in rheumatoid arthritis may be of functional interest. Somatostatin, by itself, or by interacting and antagonizing other peptides such as substance P, may regulate and influence inflammatory and repair processes. Firstly, the high density of venous somatostatin receptors may allow somatostatin to have a vasoconstrictor effect, as exerted in various parts of the body such as the mesenterium (37). Alternatively, somatostatin may counterregulate the vasodilator effect of substance P in human synovial tissue (14), and thus reduce inflammation. Secondly, somatostatin may have an effect on small blood vessels by directly altering fluid imbalance and the extravasation of leucocytes associated with inflammation, or by counterregulating the influence of substance P on the extravasation of plasma and white blood cells (38-40). Thirdly, somatostatin may also affect synovial vascular growth, since somatostatin analogues can inhibit myointimal proliferation in certain animal models (41). More generally, by affecting different stages of the inflammatory process and different tissue elements involved in inflammation, somatostatin may be part of the numerous mechanisms which determine the extent and course of this process.

Although the most widely held view is that rheumatoid arthritis is an autoimmune disease, there are numerous observations implicating the nervous system in the etiology of the disease. For instance, the important contribution of the unmyelinated afferents as well as of sympathetic efferents to neurogenic inflammation has been emphasized during the last decade (1-3). Interestingly, some of the unmyelinated afferents as well as sympathetic efferents are known to contain somatostatinergic neurons (42-44). An altered neuronal activity in these somatostatinergic pathways may lead to a disturbed synaptic release of

somatostatin which, in turn, may influence the behaviour of its vascular targets. Moreover, it has been reported that the concentration of neuropeptide degrading enzymes is increased in inflamed human synovium (45). Thus, these enzymes may lower the local concentration of somatostatin. Subsequently, the lack of local endogenous somatostatin may also affect the vascular system in rheumatoid arthritis, possibly by altering the somatostatin receptor expression.

The presence of somatostatin receptors in rheumatoid arthritis may constitute the molecular basis for therapy of rheumatoid arthritis using somatostatin analogues. Recent reports on the use of somatostatin in patients with rheumatoid arthritis suggest that such drugs may have a beneficial symptomatic effect (46). Exogenous somatostatin analogues may inhibit the inflammatory process by a direct, complex, local action through vascular somatostatin receptors, resulting in induction of vasoconstriction, inhibition of plasma extravasation, inhibition of white blood cell migration or even inhibition of neovascularization. Ultimately, such changes may lead to a reduction of pain (46). However, the possibility of an additional local and systemic action of somatostatin on the immune system (47) should also be considered, since somatostatin receptors are known to be expressed by most normal human lymphoid tissues (21,22), and since rheumatoid arthritis appears to have an autoimmune component.

The large numbers of somatostatin receptors in vessels in rheumatoid arthritis may have diagnostic consequences. Radiolabelled somatostatin analogues are currently being used successfully in the in vivo detection of somatostatin receptor positive tumours (31,32).

References

1. Levine JD, Collier DH, Basbaum AI, Moskowitz MA, Helms CA. Hypothesis: the nervous system may contribute to the pathophysiology of rheumatoid arthritis. [Review] J Rheumatol 1985;12:406.
2. Levine JD, Dardick SJ, Roizen MF, Helms C, Basbaum AI. Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. J Neurosci 1986;6:3423-9.
3. Basbaum AI, Levine JD. The contribution of the nervous system to inflammation and inflammatory disease. [Review] Can J Physiol Pharmacol 1991;69:647-51.
4. Thompson M, Bywaters EG. Unilateral rheumatoid arthritis following hemiplegia. Ann Rheum Dis 1962;21:370.

5. Glick EN. Asymmetrical rheumatoid arthritis after poliomyelitis. Br Med J 1967;3:26-8.
6. Baker GHB. Life events before the onset of rheumatoid arthritis. Psychother Psychosom 1982;38:173-7.
7. Jancso N, Jancso-Gabor A, Szolcsanyi J. Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol 1967;31:138-51.
8. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. [Review] Neuroscience 1988;24:739-68.
9. Hökfelt T, Johansson O, Ljungdahl A, Lundberg JM, Schultzberg M. Peptidergic neurones. [Review] Nature 1980;284:515-21.
10. Gronblad M, Konntinen YT, Korkala O, Liesi P, Hukkanen M, Polak JM. Neuropeptides in synovium of patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 1988;15:1807-10.
11. Walsh DA, Wharton J, Blake DR, Polak JM. Neural and endothelial regulatory peptides, their possible involvement in inflammation. [Review] Int J Tissue React 1992;14:101-11.
12. Yaksh TL. Substance P release from knee joint afferent terminals: modulation by opioids, Brain Res 1988;458:319-24.
13. Levine JD, Clark R, Devor M, Helms C, Moskowitz MA, Basbaum AI. Intraneuronal substance P contributes to the severity of experimental arthritis. Science 1984;226:547-9.
14. Pernow B. Role of tachykinins in neurogenic inflammation. J Immunol 1985;135 Suppl 2:812-5.
15. Devillier P, Weill B, Renoux M, Menkes C, Pradelles P. Elevated levels of tachykinin-like immunoreactivity in joint fluids from patients with rheumatic inflammatory diseases [letter]. N Engl J Med 1986;314:1323.
16. Marshall KW, Chiu B, Inman RD. Substance P and arthritis: analysis of plasma and synovial fluid levels. Arthritis Rheum 1990;33:87-90.
17. Walsh DA, Mapp PI, Wharton J, Rutherford RAD, Kidd BL, Revell PA, Blake DR, Polak JM. Localisation and characterisation of substance P binding to human synovial tissue in rheumatoid arthritis. Ann Rheum Dis 1992;51:313-7.
18. Wharton J, Rutherford RAD, Walsh DA, Mapp PI, Knock GA, Blake DR, Polak JM. Autoradiographic localization and analysis of endothelin-1 binding sites in human synovial tissue. Arthritis Rheum 1992;35:894-9.
19. Reichlin S. Somatostatin (first of two parts). N Engl J Med 1983;309:1495-501.
20. Reubi JC, Cortes R, Maurer R, Probst A, Palacios JM. Distribution of somatostatin receptors in the human brain: an autoradiographic study. Neuroscience 1986;18:329-46.
21. Reubi JC, Horisberger U, Waser B, Gebbers JO, Laissue JA. Preferential location of somatostatin receptors in germinal centers of human gut lymphoid tissue. Gastroenterology 1992;103:1207-14.
22. Reubi JC, Waser B, Horisberger U, Krenning EP, Lamberts SWJ, Gebbers JO, Gersbach P, Laissue JA. In vitro autoradiographic and in vivo scintigraphic localization of somatostatin receptors in human lymphatic tissue. Blood 1993;82:2143-51.
23. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. [Review] Endocr Rev 1991;12:450-82.

24. Reubi JC, Krenning EP, Lamberts SWJ, Kvols LK. In vitro detection of somatostatin receptors in human tumors. [Review] Metabolism 1992;41 Suppl 2:104-10.
25. van Hagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue JA, Hoogsteden HC, Lamberts SWJ. Somatostatin analogue scintigraphy in granulomatous diseases. Eur J Nucl Med 1994;21:497-502.
26. Reubi JC, Mazzucchelli L, Laissue JA. Intestinal vessels express a high density of somatostatin receptors in human inflammatory bowel disease. Gastroenterology 1994;106:951-9.
27. Reubi JC, Kvols LK, Waser B, Nagorney DM, Heitz PU, Charboneau JW, Reading CC, Moertel C. Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. Cancer Res 1990;50:5969-77.
28. Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
29. Reubi JC, Perrin MH, Rivier JE, Vale W. High affinity binding sites for a somatostatin-28 analog in rat brain. Life Sci 1981;28:2191-8.
30. Arnett FC, Edworthy SM, Bloch DA,. McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GH. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
31. Krenning EP, Bakker WH, Breeman WAP, Koper JW, Kooij PPM, Ausema L, Lameris JS, Reubi JC, Lamberts SWJ. Localisation of endocrine-related tumours with radioiodinated analogue of somatostatin. Lancet 1989;1:242-4.
32. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990;323:1246-9.
33. Reubi JC, Maurer R. Different ionic requirements for somatostatin receptor subpopulations in the brain. Regul Pept 1986;14:301-11.
34. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract and kidney. Proc Natl Acad Sci U S A 1992;89:251-5.
35. Yamada Y, Reisine T, Law SF, Ihara Y, Kubota A, Kagimoto S, Seino M, Seino Y, Bell GI, Seino S. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. Mol Endocrinol 1992;6:2136-42.
36. Yamada Y, Kagimoto S, Kubota A, Yasuda K, Masuda K, Someya Y, Ihara Y, Li Q, Imura H, Seino S, Seino Y. Cloning, functional expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtype. Biochem Biophys Res Commun 1993;195:844-52.
37. Sonnenberg GE, Keller U, Perruchoud A, Burckhardt D, Gyr K. Effect of somatostatin on splanchnic hemodynamics in patients with cirrhosis of the liver and in normal subjects. Gastroenterology 1981;80:526-32.
38. Lembeck F, Holzer P. Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedebergs Arch Pharmacol 1979;310:175-83.
39. Lundberg JM, Brodin E, Hua X, Saria A. Vascular permeability changes and smooth muscle contraction in relation to capsaicin-sensitive substance P afferents in the guinea-pig. Acta Physiol Scand 1984;120:217-27.

40. Sharkey KA. Substance P and calcitonin gene-related peptide (CGRP) in gastrointestinal inflammation. [Review] Ann NY Acad Sci 1992;664:425-42.
41. Lundergan CF, Foegh ML, Ramwell PW. Peptide inhibition of myointimal proliferation by angiopeptin, a somatostatin analogue. [Review] J Am Coll Cardiol 1991;17 Suppl B:132B-6B.
42. Hökfelt T, Johansson O, Luft R, Arimura A. Immunohistochemical evidence for the presence of somatostatin, a powerful inhibitory peptide, in some primary sensory neurons. Neurosci Lett 1975;1:231.
43. Hökfelt T, Elfvin LG, Elde R, Schultzberg M, Goldstein M, Luft R. Occurrence of somatostatin immunoreactivity in some peripheral sympathetic nonadrenergic neurons. Proc Natl Acad Sci U S A 1977;74:3587.
44. Sundler F, Bottcher G, Ekblad E, Hakanson R. [Review] The neuroendocrine system of the gut. Acta Oncol 1989;28:303-14.
45. Walsh DA, Mapp PI, Wharton J, Polak JM, Blake DR. Neuropeptide degrading enzymes in normal and inflamed human synovium. Am J Pathol 1993;142:1610-21.
46. Matucci-Cerinic M, Marabini S. Somatostatin treatment for pain in rheumatoid arthritis: a double blind versus placebo study in knee involvement. Med Sci Res 1988;16:233-4.
47. Stanisz AM, Scicchitano R, Stead R, Matsuda H, Tomioka M, Denburg J, Bienenstock J. Neuropeptides and immunity. [Review] Am Rev Respir Dis 1987;136:S48-51.

CHAPTER 5.3

SOMATOSTATIN RECEPTOR SCINTIGRAPHY IN SJÖGREN'S SYNDROME

Clinical aspects of Sjögren's syndrome

Sjögren's syndrome is a systemic autoimmune disease, in which predominantly the salivary and lacrimal glands are affected. Histological examination of the mucosa is characterized by mononuclear infiltrates around the salivary ducts, in which CD4 positive lymphocytes predominate. Activated CD4 positive lymphocytes are supposed to express somatostatin receptors (1). The clinical spectrum of Sjögren's disease is very heterogeneous, varying from mild complaints of dryness of the eyes or mouth to severe keratoconjunctivitis sicca, xerostomia, and extraglandular features like lung involvement. No single diagnostic test has been found for Sjögren's syndrome. There are different criteria used by various investigators to establish the diagnosis of Sjögren's syndrome. In our study the European criteria for the classification of Sjögren's syndrome were used (2). Until now no effective therapy is available. The treatment of Sjögren's syndrome remains conservative and symptomatic. Arthralgia and myalgia are generally treated with simple non-steroidal antiinflammatory drugs (NSAIDs) or antimalarials. NSAIDs and low dose corticosteroids are effective for systemic symptoms like fever and weariness. High dosages of corticosteroids and immunosuppressive drugs may be considered in case of severe extraglandular manifestations with life threatening organ failure (3).

Somatostatin receptor scintigraphy in Sjögren's syndrome

In chapter 2 the somatostatin receptor expression by activated lymphocytes is described. Recently, we performed somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^{1}]-octreotide, in 12 patients with Sjögren's syndrome, in order to detect somatostatin receptor expression in the affected tissue. In nine patients uptake of radioactivity was found in the affected salivary glands (Figure 1 and Figure 2), lungs, joints, and even in the heart. Interestingly, the uptake in the region of the lung decreased during treatment with hydroxychloroquine, which is supposed to be stored in the lungs.

Somatostatin receptor scintigraphy enables the visualization of the extent of disease in Sjögren's syndrome, and may be used in the monitoring of the efficacy of therapy, especially in the case of lung involvement.

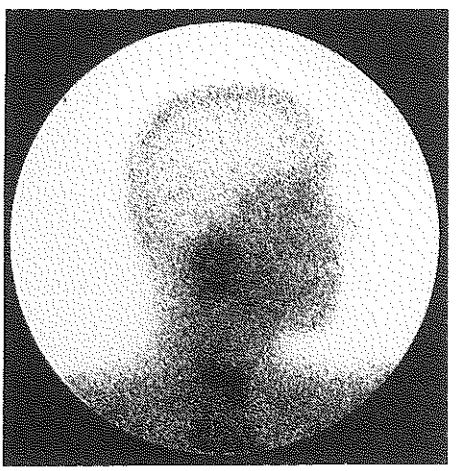

Figure 1. Visualization of the salivary gland in a patient with Sjögren's syndrome by ^{111}In labelled somatostatin analogue scintigraphy (lateral view, 24 hours after injection).

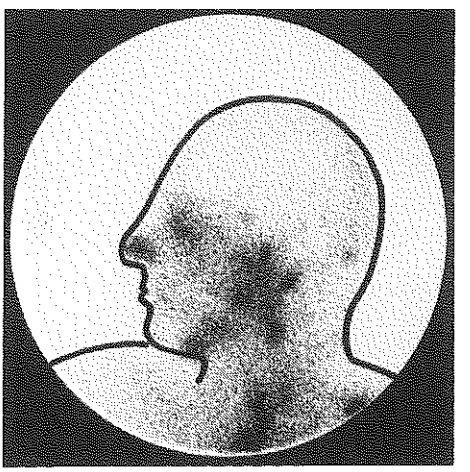

Figure 2. Visualization of the left lacrimal gland, parotid gland, submaxillary gland and associated lymphadenopathy in a patient with Sjögren's syndrome (lateral view, 24 hours after injection).

References

1. van Hagen PM, Krenning EP, Kwekkeboom DJ, Reubi JC, van den Anker-Lugtenburg PJ, Löwenberg B, Lamberts SWJ. Somatostatin in the immune and haematopoetic system; a review. Eur J Clin Invest 1994;24:91-9.
2. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, Bjerrum KB, Braga S, Coll J, de Vita S, et al. Preliminary criteria for the classification of Sjögren's syndrome. Arthritis Rheum 1993;36:340-7.
3. Fox RI. Treatment of the patient with Sjögren's syndrome. Rheum Dis Clin North Am 1992;18:699-709.

CHAPTER 6

SOMATOSTATIN RECEPTOR SUBTYPE EXPRESSION IN HUMAN LYMPHOID CELL LINES

Abstract

Previous studies have shown that human lymphoid cells express somatostatin receptors. Recently, five somatostatin receptor subtypes have been identified. In this study the expression of messenger ribonucleic acid (mRNA) for somatostatin receptor subtypes was investigated in ten B cell lines and eight T cell lines by reverse transcriptase polymerase chain reaction (RT-PCR). Two B cell lines (JY and TMM) and two T cell lines (HSB-2 and MT-1) expressed mRNA for somatostatin receptor subtype 2, resulting in the expression of membrane somatostatin receptors in the cell lines JY, TMM and MT-1. We therefore conclude that only somatostatin receptor subtype 2 mRNA is expressed in human lymphoid cells.

Introduction

Somatostatin receptor expression in non-activated peripheral lymphocytes and monocytes was first described by Bhathena et al. (1). Several groups have reported the expression of somatostatin receptors by human and animal white blood cells (2). Recently, somatostatin receptor subtype 2 was found in *Schistosoma mansoni* egg induced granulomas in mice (3). Furthermore, in situ hybridization showed the expression of somatostatin receptor subtype 2 in Hodgkin's lymphomas and of somatostatin receptor subtypes 2 and 3 in non-Hodgkin's lymphomas (4). In the immune system, the density of somatostatin receptors is low, which makes the identification of somatostatin receptors on single cells or tissue sections extremely difficult.

In this report we present our results concerning the detection of mRNA encoding for five somatostatin receptor subtypes in lymphoid cell lines by a subtype specific RT-PCR. Ten human lymphoid B cell lines and eight human lymphoid T cell lines were investigated for somatostatin receptor mRNA expression in relation to their haematopoietic differentiation state.

Materials and methods

Cell lines

Eighteen lymphoid cell lines were investigated (Table 1 and Table 2). The T cell

Table 1. B cell lines.

Pre B cells	Immature B cells	Mature B cells / plasma cells
RS4,11a	Raji	TMM
Nalm-1	Daudi	LP-1
BV-173	JY	L-363
Nalm-6		

Table 2. T cell lines.

CD3 negative cells	TcR-δ positive cells	TcR-αß positive cells
HSB-2	Peer	Molt-16
Molt-3		HuT-78
ALL-1		HPB-ALL
MT-1		

lines were characterized by T cell receptor (TcR) gene rearrangement as described previously (5).

RNA isolation

Total cytoplasmic RNA was isolated according to standard procedures described by Chomczynski et al. (6). Briefly, cells (5-10 x 10^6) were homogenized with 0.5 ml of solution D in a 1.5 ml Eppendorf tube. Sequentially, 50 µl of 2 M sodium acetate (pH 4.0), 0.5 ml of water saturated phenol, and 0.1 ml of chloroform were added to the homogenate, with thorough mixing after the addition of each reagent. The final suspension was cooled on ice for 15 minutes. Samples were centrifuged at 10,000 x g for 15 minutes at 4°C. The aqueous phase was transferred to a fresh tube containing 0.6 ml of isopropanol, and then placed at 4°C for at least 6 hours to precipitate RNA. RNA was recovered by centrifugation (10,000 x g, 15 minutes, 4°C), dissolved in 0.3 to 0.5 ml solution D, and precipitated again with 1 volume of isopropanol. After centrifugation (10,000 x g, 15 minutes, 4°C), the RNA pellet was washed in 0.6 to 1.0 ml 75% ethanol and eventually dissolved in 25-50 µl RNase-free water. From 10 µg of total cytoplasmic RNA, polyA$^+$ mRNA was isolated using paramagnetic oligo-(dT) beads (Dynabeads Oligo $(dT)_{25}$, Dynal AS, Oslo, Norway) according to the manufacturers instructions. For each polyA$^+$ mRNA

isolation 30 µl of the oligo-(dT)-beads suspension was used in a volume of 200 µl and the polyA$^+$ mRNA was finally eluted in 20 µl 2 mM EDTA, pH 8.0.

Synthesis of complementary deoxyribonucleic acid (cDNA)
cDNA was synthesized from 2 µl of the isolated polyA$^+$ mRNA by using AMV Reverse Transcriptase (SuperRT, HT Biotechnology Ltd, Cambridge, United Kingdom), under conditions described by the manufacturer, and 5 pmol oligo-$(dT)_{12-18}$ primer (Gibco BRL, Renfrewshire, Scotland) (7). Each cDNA reaction was performed in duplicate.

Polymerase chain reaction (PCR)
One-thirteenth of the synthesized cDNA was used for each PCR reaction. The 50 µl reaction mixtures contained, in addition to the DNA template, 0.1 units SuperTaq DNA polymerase (HT Biotechnology Ltd, Cambridge, United Kingdom), buffer provided with the enzyme, 50 µM of each of the four deoxynucleotides dATP, dCTP, dGTP and dUTP (HT Biotechnology Ltd, Cambridge, United Kingdom), and 5 pmol of forward and reverse primers specific for one of the five somatostatin receptor subtypes or for the ß actin gene. With each of the duplicate cDNA samples PCR reactions were performed for all primer pairs. The primers specific for somatostatin receptor subtype 1 and 2 were described by Wulfsen et al. (8), the primers for receptor subtype 3, 4 and 5 by Kubota et al. (9) and the ß actin primers by Vidal et al. (10). The reactions were performed in a DNA thermal cycler (Perkin Elmer Cetus Instruments, Gouda, the Netherlands). After an initial denaturation for 5 minutes at 94°C, the samples were subjected to 35 cycles of denaturation, annealing and extension of 1 minute at 94°C, 2 minutes at 59°C and 1 minute at 72°C, respectively. After a final extension of 10 minutes at 72°C, 10 µl aliquots of the resulting PCR products were analyzed on 1% agarose gels.

Controls
To ascertain that no DNA was present in the polyA$^+$ mRNA preparations, the cDNA reactions were also performed once without reverse transcriptase, and amplified with primers specific for somatostatin receptor subtype 1. Amplification of the duplicate cDNA samples with the ß actin specific primers served as a positive control for the quality of the cDNA. To exclude contamination of the PCR reaction mixtures the reactions were also performed in the absence of DNA template in parallel with the cDNA samples. Plasmid DNA, containing the

genes for receptor subtypes 1-5, was amplified in parallel with the cDNA samples as a positive control for the PCR. These plasmids were a generous gift of Dr. G.I. Bell (Howard Hughes Medical Institute Research Laboratories, University of Chicago, Chicago, Illinois, USA). The plasmids were used in dilutions down to 0.01 pg per PCR reaction, which gives a weak but consistent signal when analyzed on gel. This amount of plasmid DNA contains approximately 3000 copies of the template. Assuming that 10 μg of total cytoplasmic RNA represents the amount of RNA of 10^6 cells (10^{-5} μg RNA per cell), and that the combined efficiencies of the polyA$^+$ mRNA isolation with the oligo-(dT) beads and the cDNA reaction is in the order of 50%, approximately 4000 copies of template would be expected in the amount of cDNA used in the PCR reactions, if the cells contained 1 mRNA molecule per cell. Therefore, signals that were consistently weaker than the signal produced by amplification of 0.01 pg plasmid DNA were considered not to be biologically relevant, and were depicted as negative result.

Ligand binding assay

Reaction conditions were the same as described by Reubi et al. (11). The radioligand used in the binding studies was the [^{125}I-Tyr^3]-octreotide (Sandoz, Basel, Switzerland). Briefly, membrane preparations (corresponding to 15-30 μg protein) of cells cultured for 14 days (JY cell line), 18 days (TMM and HSB-2 cell lines) or 32 days (MT-1 cell line) in RPMI without HEPES (Gibco BRL, Renfrewshire, Scotland), with 10% foetal calf serum were incubated in a total volume of 100 μl at room temperature for 90 minutes with radioligand (30,000-50,000 cpm) and increasing concentrations of unlabelled [Tyr^3]-octreotide in HEPES buffer (10 mM HEPES, 5 mM $MgCl_2$ and 0.2 g/l bacitracin, pH 7.6) containing 0.2% bovine serum albumin. After the incubation, 1 ml ice cold HEPES buffer was added to the assay mixture, and membrane bound radioactivity was separated from unbound by centrifugation during 2 minutes at 14,000 rpm in an Eppendorf microcentrifuge (12). The remaining pellet was washed twice with ice cold HEPES buffer, and the final pellet was counted in a gamma counter. Specific binding was taken to be the total binding minus the binding in the presence of 10 μM unlabelled [Tyr^3]-octreotide. Unrelated compounds, such as thyrotropin releasing hormone, luteinizing hormone-releasing hormone, and epidermal growth factor, added in a 1000-fold excess were not able to displace [^{125}I-Tyr^3]-octreotide binding.

Results

All cell lines were investigated with RT-PCR for the expression of human somatostatin receptor mRNA (subtypes 1-5). The results are shown in Table 3 and Table 4. The two B cell lines JY and TMM and the two T cell lines HSB-2 and MT-1 were found to express mRNA for somatostatin receptor subtype 2. No mRNA for the other receptor subtypes was detected in these human lymphoid cell lines. The expression of mRNA for somatostatin receptor subtype 2 in the two human B cell lines was in cells of the immature B cell range. Somatostatin receptor subtype 2 mRNA in the T cell lines was found in the CD3 negative 'early' differentiation range. It should be mentioned, however, that not all immature and mature B cell lines tested were somatostatin receptor subtype 2 mRNA positive, while only two of the four CD3 negative T cell lines tested expressed the somatostatin receptor subtype 2 mRNA.

Scatchard analyses with [^{125}I-Tyr^{3}]-octreotide revealed high affinity binding sites in the TMM, JY, and MT-1 cell lines (Figure 1). Ligand binding analyses with [^{125}I-Tyr^{3}]-somatostatin 28 showed identical results, indicating that only somatostatin receptor subtype 2 was expressed (data not shown). The presence of somatostatin receptor subtype 2 mRNA in the HSB-2 cell line did not result in the expression of this somatostatin receptor subtype in the cellular membrane.

Discussion

Radioligand binding studies as well as biochemical and physiological evidence suggested the existence of at least two somatostatin receptor subtypes (13,14). Recently, somatostatin receptor heterogeneity was further demonstrated by molecular cloning of a family of five putative somatostatin receptors (15-23). Somatostatin receptors are expressed by human lymphoid cell lines, normal lymphocytes as well as malignant lymphoid cells (2). Previously, specific somatostatin binding sites were found on the human T cell leukaemia cell line MT-2, the Epstein-Barr virus transformed B cell line Isk, and the human lymphoblastic cell line Molt-4 (24). They were also found in human thymus and spleen, and were identified within the germinal centres of gastrointestinal associated lymphoid tissue (25,26). These observations led to the suggestion that somatostatin receptor expression is related to the proliferative state of

Table 3. Detection of messenger ribonucleic acid (mRNA) for somatostatin receptors in lymphoid B cell lines by reverse transcriptase polymerase chain reaction (RT-PCR).

	Somatostatin receptor subtype				
	1	2	3	4	5
Pre B cell					
RS4,11a	-	-	Not done	Not done	Not done
Nalm-1	-	-	-	-	-
BV-173	-	-	-	-	-
Nalm-6	-	-	-	-	-
Immature B cell					
Raji	-	-	-	-	-
Daudi	-	-	-	-	-
JY	-	+	-	-	-
Mature B cell / plasma cell					
TMM	-	+	-	-	-
LP-1	-	-	-	-	-
L-363	-	-	-	-	-

lymphoid cells. Recently, somatostatin receptor subtypes 2 and 3 were found in malignant lymphomas with in situ hybridisation techniques (somatostatin receptor subtypes 4 and 5 were not investigated) (4).

In our scintigraphic studies with [^{111}In-DTPA-D-Phe^{1}]-octreotide, radioactivity was localized in 'activated' or malignant lymph nodes while normal lymph nodes were negative. This radiopharmaceutical, which is strongly related to octreotide, binds with high affinity to human somatostatin receptor subtype 2 and 5 and with low affinity to receptor subtype 3 (27).

In the literature different techniques have been reported for the detection of somatostatin receptors in the immune system, such as autoradiography, ligand binding assays, and fluorescent techniques using a fluorescent labelled somatostatin analogue (2). In our hands, no reproducible binding experiments with avidin-biotin labelled somatostatin analogues could be obtained. Fluorescent techniques may be too insensitive for the detection of low numbers of somatostatin receptors on individual cells.

Table 4. Detection of messenger ribonucleic acid (mRNA) for somatostatin receptors in lymphoid T cell lines by reverse transcriptase polymerase chain reaction (RT-PCR).

	Somatostatin receptor subtype				
	1	2	3	4	5
CD3 negative cell					
HSB-2	-	+	-	-	-
Molt-3	-	-	-	-	-
ALL-1	-	-	-	-	-
MT-1	-	+	-	-	-
TcR-δ positive cell					
Peer	-	-	-	-	-
TcR-αß positive cell					
Molt-16	-	-	-	-	-
HuT-78	-	-	-	-	-
HPB-ALL	-	-	-	-	-

In this study mRNA for somatostatin receptor subtype 2 was found in two B- and two T cell lines without expression of other somatostatin receptors, suggesting that somatostatin receptor expression in human lymphoid cells is restricted to receptor subtype 2. In the B cell lines the immature (JY) and mature (TMM) differentiated cell lines were somatostatin receptor subtype 2 mRNA positive; in the T cell lines the cytoplasmic CD3 negative ('early') T cell lines MT-1 and HSB-2 were positive.

Somatostatin receptors were found in vitro and in vivo in patients with specific autoimmune diseases and with malignant lymphomas (2), while no somatostatin receptor expression was found in non-activated lymph nodes in patients by somatostatin receptor scintigraphy. These results suggest that somatostatin receptor expression may be related to the activation/differentiation state of lymphoid cells. Somatostatin receptor subtype 2 specific agents, like octreotide, RC-160 and BIM-23014 may be potential tools to influence the differentiation and/or proliferation of activated and malignant lymphoid cells.

Clearly, more primary human tissues of autoimmune diseases and malignant lymphomas need to be investigated for their expression of somatostatin receptor subtypes, in order to establish whether the conclusions drawn from our study

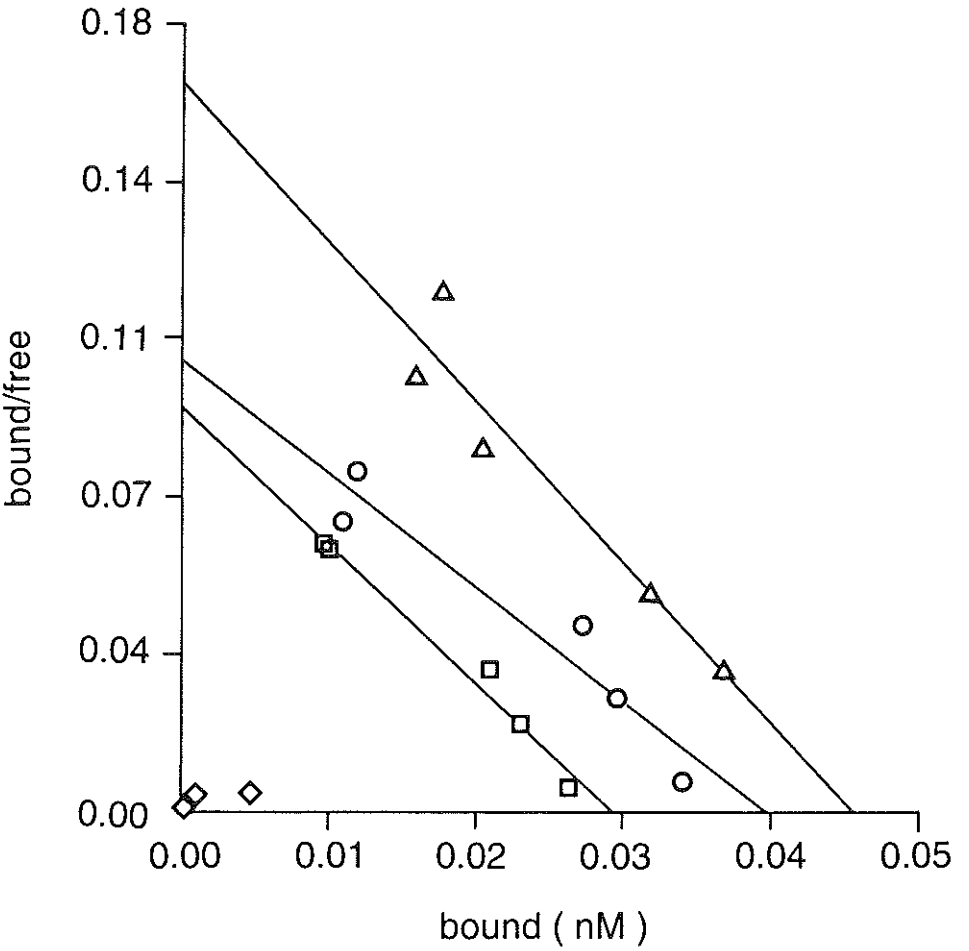

Figure 1. Scatchard analysis of [^{125}I-Tyr^{3}]-octreotide binding on human T and B cell lines. □ JY cell line (K_d = 0.3 nM,n = 186 fmoles/mg), ○ TMM cell line (K_d = 0.4 nM, n = 206 fmoles/mg), ◇ HSB-2 cell line (not detectable), △ MT-1 cell line (K_d = 0.3 nM, n = 332 fmoles/mg).

in cell lines are representative for the somatostatin receptor status in vivo in the abovementioned diseases.

References

1. Bhathena SJ, Louie J, Schechter GP, Redman RS, Wahl L, Recant L. Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. Diabetes 1981;30:127-31.
2. van Hagen PM, Krenning EP, Kwekkeboom DJ, Reubi JC, van den Anker-Lugtenburg PJ, Löwenberg B, Lamberts SWJ. Somatostatin and the immune and haematopoetic system; a review. [Review] Eur J Clin Invest 1994;24:91-9.
3. Elliott DE, Metwali A, Blum AM, Sandor M, Lynch R, Weinstock JV. T lymphocytes isolated from the hepatic granulomas of schistosome-infected mice express somatostatin receptor subtype II (SSTR2) mesenger RNA. J Immunol 1994; 153:1180-6.
4. Reubi JC, Schaer JC, Waser B, Mengod G. Expression and localization of somatostatin receptor SSTR1, SSTR2, and SSTR3 messenger RNAs in primary human tumors using in situ hybridisation. Cancer Res 1994;54:3455-9.
5. van Dongen JJM. Human T cell differentiation. Basic aspects and their clinical applications [dissertation]. Rotterdam: Erasmus University Rotterdam, 1990.
6. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
7. Sambrook J, Fritch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
8. Wulfsen I, Meyerhof W, Fehr S. Richter D. Expression patterns of rat somatostatin receptor genes in pre- and postnatal brain and pituitary. J Neurochem 1993;61:1549-52.
9. Kubota A, Yamada Y, Kagimoto S, Shimatsu A, Imamura M, Tsuda K, Imura H, Seino S, Seino Y. Identification of somatostatin receptor subtypes and an implication for the efficacy of somatostatin analogue SMS 201-995 in treatment of human endocrine tumors. J Clin Invest 1994;93:1321-5.
10. Vidal C, Rauly I, Zeggari M, Delesque N, Esteve JP, Saint-Laurent N, Vaysse N, Susini C. Up-regulation of somatostatin receptors by epidermal growth factor and gastrin in pancreatic cancer cells. Mol Pharmacol 1994;46:97-104.
11. Reubi JC. New specific radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
12. Viguerie N, Tahiri-Jouti N, Ayral M, Cambillau C, Scemama JL, Bastie MJ, Knuhtsen S, Esteve JP, Pradayrol L, Susini C, Vaysse N. Direct inhibitory effects of a somatostatin analog, SMS 201-995, in AR4-2J cell proliferation via pertussis toxin-sensitive guanosine triphosphate-binding protein-independent mechanism. Endocrinology 1989;124:1017-25.
13. Srikant CB, Patel YC. Receptor binding of somatostatin-28 is tissue specific. Nature 1981;294:259-60.
14. Reubi JC. Evidence of two somatostatin-14 receptor types in rat brain cortex. Neurosci Lett 1984;49:259-63.
15. Yasuda K, Rens-Domiano S, Breder CD, Law SF, Saper CB, Reisine T, Bell GI. Cloning of a novell somatostatin receptor, SSTR3, coupled to adenylylcyclase. J Biol Chem 1992;267:20422-8.
16. Yamada Y, Post SR, Wang K, Tager HS, Bell GI, Seino S. Cloning and functional characterization of a family of human and mouse somatostatin receptors expressed in brain, gastrointestinal tract, and kidney. Proc Natl Acad Sci U S A 1992;89:251-5.

17. Kluxen F, Bruns C, Lubbert H. Expression cloning of a rat brain somatostatin receptor cDNA. Proc Natl Acad Sci U S A 1992;89:4618-22.
18. Bruno JF, Xu Y, Song J, Berelowitz M. Molecular cloning and functional expression of a brain specific somatostatin receptor. Proc Natl Acad Sci U S A 1992:89;11151-5.
19. O'Carroll AM, Lolait SJ, Konig M, Mahan LC. Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity to somatostatin-28. Mol Pharmacol 1992;42:939-46.
20. Li XJ, Forte M, North RA, Ross CA, Snyder SH. Cloning and expression of a rat somatostatin receptor enriched in brain. J Biol Chem 1992;267:21307-12.
21. Yamada Y, Reisine T, Law SF, Ihara Y, Kubota A, Kagimoto S, Seino M, Seino Y, Bell GI, Seino S. Somatostatin receptors, an expanding gene family: cloning and functional characterization of human SSTR3, a protein coupled to adenylyl cyclase. Mol Endocrinol 1992;6:2136-42.
22. Rohrer L, Raulf F, Bruns C, Buettner R, Hofstaedter F, Schule R. Cloning and characterization of a fourth somatostatin receptor. Proc Natl Acad Sci U S A 1993;90:4196-200.
23. Panetta R, Greenwood MT, Warszynska A, Demchyshin LL, Day R, Niznik HB, Srikant CB, Patel YC. Molecular cloning, functional characterization, and chromosomal localization of a human somatostatin receptor (somatostatin receptor type 5) with preferential affinity for somatostatin-28. Mol Pharmacol 1993;45:417-27.
24. Nakamura H, Koike T, Hiruma K, Sato T, Tomioka H, Yoshida S. Identification of lymphoid cell lines bearing receptors for somatostatin. Immunology 1987;62:655-8.
25. Reubi JC, Horisberger U, Waser B, Gebbers JO, Laissue JA. Preferential location of somatostatin receptors in germinal centers of human gut lymphoid tissue. Gastroenterology 1992;103:1207-14.
26. Reubi JC, Waser B, Horisberger U, Krenning EP, Lamberts SWJ, Gebbers JO, Gersbach P, Laisseu JA. In vitro autoradiographic and in vivo scintigraphic localization of somatostatin receptors in human lymphatic tissue. Blood 1993;82:2143-51.
27. Weckbecker G, Raulf F, Stolz B, Bruns C. Somatostatin analogs for diagnosis and treatment of cancer. [Review] Pharmacol Ther 1993;60:245-64.
28. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T-lymphocytes and Molt-4 lymphoblasts. Cell Immunol 1984;84:433-8.

CHAPTER 7

AN INTRODUCTION TO SUBSTANCE P

Substance P

Substance P is a peptide of eleven amino acids (Figure 1), discovered by Euler et al. (1), and chemically characterized many years later. It was initially found to be present in very high concentrations in the substantia nigra (1-4). Substance P is a member of the family of tachykinins. Tachykinins are neuropeptides that are synthesized primarily by neurons, and share a common C terminal amino acid sequence (-Phe-X-Gly-Leu-Met-NH_2).

Currently the mammalian tachykinin family is known to be composed of three neuropeptides, i.e. substance P, neurokinin A and neurokinin B. The neurokinin 1 receptor, which is present in both the central and peripheral nervous system, preferentially interacts with substance P, as substance P has the highest affinity (K_d = 0.5-1.0 nM) for this receptor. The affinities of neurokinin A and neurokinin B for the neurokinin 1 receptor are generally two- to fourfold lower (5-7). The neurokinin 1 receptor has recently been cloned and was shown to belong to the family of G protein coupled receptors (8,9).

Substance P is found in the peripheral sensory nerves, which innervate organs and tissues, such as the gastrointestinal and respiratory tract, the visual system, the skin and, relevant to the immune system, the lymph nodes and the spleen (10). Tachykinin receptors have a wide tissue distribution. Interaction between the tachykinins and their receptors is associated with diverse responses, such as sensory transmission, both contraction and endothelium dependent relaxation of vascular smooth muscle, nociception, histamine release, plasma extravasation, inflammation and, potentially, nerve regeneration and wound healing (11).

Substance P and the immune system

Substance P modulates several immune and hypersensitivity activities. It has been reported to modulate the functional activities of inflammatory cells by causing degranulation of mast cells (but not basophils), stimulation of lysosomal enzyme release from neutrophils, enhancement of neutrophil chemotaxis and phagocytosis, and activation of macrophages (11-15). Chemotaxis and lysosomal secretion, induced by substance P, operate via cross reactivity with the receptor for the chemotactic peptide N-formylmethionyl-leucyl-phenylalanine

Substance P
Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met

Figure 1. Sequence of substance P.

(fMet-Leu-Phe). Substance P has also been shown to stimulate T lymphocyte proliferation (60-70%), as measured by [^{3}H]-thymidine incorporation, to enhance the effect of T cell mitogens and the production of immunoglobulins by plasma cells (16-19). The proliferative effects of substance P reside in the C terminal end of the molecule. Some investigators were unable to obtain any mitogenic effect of substance P or to detect substance P receptors on human peripheral blood lymphocytes, however (20). Evidence was found that substance P binds to a non-neurokinin 1 receptor in monocytes, which activates a mitogen activated protein kinase (20).

Substance P binds to specific receptors on rat tissue mast cells to induce histamine and leukotriene release at micromolar concentrations (11). It has been suggested that the C terminal portion of substance P is required for binding to the receptor and that the N terminal basic amino acids are required for cell activation (21). The hypersensitivity effects, such as vasodilatation and alteration of the microvascular permeability appear to be mediated by substance P induced factors such as histamine and leukotrienes. In contrast to the activation of tissue mast cells to release mediators, substance P has no effect on basophils (11). Thus, the hypersensitivity effects of substance P are cell type specific.

There are several reports showing that substance P is involved in cytokine production. Substance P and 12-phorbol-12-myristate-13-acetate synergistically and dose dependently induce interleukin 2 production in murine EL-4 thymoma cells and human peripheral blood cells (22,23). Substance P stimulates the production of interleukin 1, tumour necrosis factor α, and interleukin 6 (24). On the other hand interleukin 1 increases the level of substance P in cultured neonatal superior cervical ganglions (25). Recently, it has been shown that substance P selectively activates gene expression and secretion of tumour necrosis factor α in murine mast cells (26). Substance P is present in nerve fibres that innervate the spleen, thymus and lymph nodes, and in nerve fibres that are in close contact with the mast cells in the intestine. This supports the

concept that substance P influences the activity of the immune system (27-29).

Substance P in inflammatory conditions

Several lines of evidence indicate that tachykinin neuropeptides like substance P play a role in the inflammatory and immune response, both in peripheral tissues, such as the lungs, joints and colon, as well as in the central nervous system. Terminals of substance P containing sensory nerves were observed in association with blood vessels. Electric stimulation of these peripheral nerves at intensities that stimulate substance P release reproduces many of the physiological changes that are seen in acute inflammation. In recent years, the focus of attention has shifted from the central nervous system neurons themselves, which appear to have the capacity to regenerate, to the central nervous system glia, which apparently inhibits the regrowth of axons in the central nervous system. It has been demonstrated that, after injury, regenerating axons grow a short distance until they reach the glial scar, where they appear to stop growing and degenerate (30). The major cellular constituent of a central nervous system glial scar is the reactive astrocyte (astrocytes originate in the monocyte lineage) (31). In the normal optic nerve tract no substance P receptors are expressed, but after injury binding sites for substance P are expressed by the glial scar (32). These results demonstrate that glial cells in vivo express high concentrations of substance P receptors after transection of retinal ganglion cell axons.

Substance P receptors are expressed at high concentrations in arterioles, venules and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease (33). In the normal colon a low concentration of substance P binding sites is expressed by submucosal arterioles and venules, and a moderate level is expressed by the external circular muscle. These findings reveal that in a disease state a specific tachykinin binding site for substance P appears to be overexpressed by specific cells. A key question is whether substance P receptors are an etiological factor, or whether they are concomitant with inflammation. Corticosteroids do not downregulate the substance P receptors, suggesting a more etiological role for substance P in these diseases. In ulcerative colitis and Crohn's disease the number of substance P- and vasoactive intestinal peptide positive nerves decreased in severe inflammatory lesions (34). A difference in the substance P

concentration was found between ulcerative colitis and Crohn's disease (35). The substance P concentration in rectal biopsies from patients with ulcerative colitis was increased, when compared with control patients and patients with Crohn's disease. Thus, the role of substance P in the pathogenesis of ulcerative colitis and Crohn's disease may be different.

Clinical evidence for the involvement of the nervous system in arthritis came from paralysed patients. In patients with upper motor neuron hemiplegia or lower motor neuron lesions, such as poliomyelitis, no symptoms of any subsequent development of rheumatoid arthritis were found in the affected limbs (36,37). Evidence from animal studies showed that sectioning of one sciatic nerve, seven days before the induction of adjuvant arthritis in the rat, delayed the onset and reduced the severity of disease in the operated limb (38). However, the opposite finding was reported by another group (39). Another indication of nervous involvement is the exact symmetry of a number of arthritic conditions (40). In rheumatoid arthritis synovia samples, a decrease in substance P containing nerve fibres was found, with a simultaneous increase in substance P receptor density (41). It was suggested that activation of nociceptors by chemical stimuli, for example prostaglandins, might have caused an increased release of substance P and therewith a lowering of the substance P content (41). Substance P concentrations in serum and synovial fluid in patients with rheumatoid arthritis are increased. Lymphocytes, obtained from the peripheral blood and the synovial fluid from patients with rheumatoid arthritis, were not responsive to exogenous substance P, in contrast to peripheral blood lymphocytes from control patients (42). Lymphocyte receptors for substance P may be desensitized because of large amounts of the peptide found in situ (42). Repeated stimulation with substance P causes a typical tachyphylaxis in many systems (43). Substance P is capable of stimulating synoviocytes, which enhances pannus formation and increases connective tissue damage (44). Substance P does not accumulate in significant amounts within the synovial fluid (45). In synovial tissue a high expression of the enzyme neural endopeptidase was found in cells surrounding the blood vessels compared with normal human synovium (46). This finding suggests an increase in the metabolism of tachykinins. Intraarticular injections of substance P in rat knee joints resulted in a pronounced inflammatory response (47). Substance P perfusion of an inflamed joint was shown to exacerbate the inflammation and enhance extravasation of plasma proteins into the synovial cavity (48). It has also been demonstrated that the number of substance P receptors in rheumatoid synovium

is largely increased when compared with normal synovium (49,50). The highest concentrations of these receptors are present in blood vessels, on endothelial lining cells, and on the surrounding smooth muscle cells. It is now widely accepted that there is a neurogenic mechanism in the pathophysiology of arthritis, and substance P may be one of the important mediators.

References

1. Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. J Physiol 1931;72:74-87.
2. Pernow B. Studies on substance P: purification, occurrence and biological actions. Acta Physiol Scand 1953;105:1-90.
3. Lembeck F, Starke K. Substance P content and effect on capillary permeability of extract of various parts of human brain. Nature 1963;199:1295-6.
4. Chang MM, Leeman SE, Niall HD. Amino acid sequence of substance P. Nature New Biol 1971; 232:86-7.
5. Torrens Y, Beaujouan JC, Glowinski J. Pharmacological characterisation of two tachykinin binding sites in the rat cerebral cortex. Neuropeptides 1985;6:59-70.
6. Mantyh PW, Gates T, Mantyh CR, Maggio JE. Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J Neurosci 1989;9:258-79.
7. Mantyh PW, Vigna SR, Maggio JE. Assays for substance P and tachykinin receptors. Methods Neurosci 1991;5:404-25.
8. Hershey AD, Krause JE. Molecular characterization of a functional cDNA encoding the rat substance P receptor. Science 1990;247:958-62.
9. Hershey AD, Dykema PE, Krause JE. Organization, structure and expression of the gene encoding the rat substance P receptor. J Biol Chem 1991;266:4366-74.
10. Pernow B. Substance P. [Review] Pharmacol Rev 1983;35:85-141.
11. Goetzl EJ, Chernov T, Renold F, Payan DG. Neuropeptide regulation of the expression of immediate hypersensitivity. J Immunol 1985;135 Suppl:S802-5.
12. Bar-shavit Z, Goldman R, Stabinsky Y, Gottlieb P, Fridkin M, Teichberg VI, Blumberg S. Enhancement of phagocytosis - a newly found activity of substance P residing in its N-terminal tetrapeptide sequence. Biochem Biophys Res Commun 1980;94:1445-51.
13. Marasco WA, Showell HJ, Becker EL. Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. Biochem Biophys Res Commun 1981;99:1065-72.
14. Mazurek N, Pecht I, Teichberg VI, Blumberg S. The role of the N-terminal tetrapeptide in the histamine releasing action of substance P. Neuropharmacology 1981;20:1025-7.
15. Hartung HP, Wolters K, Toyka KV. Substance P: binding properties and studies on cellular responses in guinea pig macrophages. J Immunol 1986;136:3856-63
16. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983:131;1613-5.

17. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
18. Reusch MK, Karasek MA, Nickoloff BJ. Effect of neuropeptides present in skin on the proliferation of human peripheral mononuclear cells and T cells. Arch Dermatol Res 1988;280:279-81.
19. Roberts AI, Taunk J, Eberts EC. Human lymphocytes lack substance P receptors. Cell Immunol 1992;141:457-65.
20. Jeurissen FJF. The tachykinin substance P; receptor-G protein coupling in mononuclear cells [dissertation]. Utrecht: University of Utrecht, 1994.
21. Foreman J, Jordan C. Histamine release and vascular changes induced by neuropeptides. Agents Actions 1983;13:105-16.
22. Rameshwar P, Gascon P, Ganea D. Immunoregulatory effects of neuropeptides. Stimulation of interleukin-2 production by substance P. J Neuroimmunol 1992;37:65-74.
23. Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T cells. J Immunol 1992;148:3498-504.
24. Lotz M, Vaughan JH, Carson DA. Effects of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988;241:1218-21.
25. Jonakait GM, Schotland S, Hart RP. Effects of lymphokines on substance P in injured ganglia of the peripheral nervous system. [Review] Ann N Y Acad Sci 1991;632:19-30.
26. Ansel JC, Brown JR, Payan DG, Brown MA. Substance P selectively activates TNF-alpha gene expression in murine mast cells. J Immunol 1993;150:4478-85.
27. Weihe E, Muller S, Fink T, Zentel HJ. Tachykinins, calcitonin gene-related peptide, and neuropeptide Y in nerves in the mammalian thymus: interaction with mast cells in autonomic and sensory neuroimmunomodulation? Neurosci Lett 1989;100:77-82.
28. Bellinger DL, Lorton D, Romano TD, Olschowka JA, Felten SY, Felten DL. Neuropeptide innervation of lymphoid organs. [Review] Ann N Y Acad Sci 1990;594:17-33.
29. Bienenstock J, Tomioka M, Matsuda H, Stead RH, Quinonez G, Simon GT, Coughlin MD, Denburg JA. The role of mast cells in inflammatory processes: evidence of nerve/mast cell interactions. Int Arch Allergy Appl Immunol 1987;82:238-43.
30. Liuzzi FJ, Lasek RJ. Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway. Science 1987;237:642-5.
31. Miller RH, Abney ER, David S, French-Constant C, Lindsay R, Patel R, Stone JR, Raff MC. Is reactive gliosis a property of a distinct subpopulation of astrocytes? J Neuroscience 1986;6:22-9.
32. Mantyh PW, Johnson DJ, Boehmer CG, Catton MD, Vinters HV, Maggio JE, Too HP, Vigna SR. Proc Natl Acad Sci U S A 1989;86:5193-7.
33. Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EP Jr., Vigna SR, Maggio JE, Kruger L, Mantyh PW. Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn disease. Proc Natl Acad Sci U S A 1988;85;3235-9.

34. Kimura M, Masuda T, Hiwatashi N, Toyota T, Nagura H. Changes in neuropeptide-containing nerves in human colonic mucosa with inflammatory bowell disease. Pathol Int 1994;44:624-34.
35. Bernstein CN, Robert ME, Eysselein VE. Rectal substance P concentrations are increased in ulcerative colitis but not in Crohn's disease. Am J Gastroenterol 1993;88:908-13.
36. Thompsom M, Bywaters EGL. Unilateral rheumatoid arthritis following hemiplegia. Ann Rheum Dis 1961:21;370-7.
37. Glick EN. Asymmetrical rheumatoid arthritis after poliomyelitis. Br Med J 1967;3:26-8.
38. Courtright LJ, Kuzell WC. Sparing effect of neurological deficit and trauma on the course of adjuvant arthritis in rats. Ann Rheum Dis 1965;24:360-7.
39. Rees RG, Dixey JJ, Lightman SL, Brewerton DA. The influence of unilateral nerve section on the course of adjuvant arthritis in rats. Arthritis Rheum 1989;32 Suppl:S54.
40. Kidd BL, Mapp PI, Gibson SJ, Polak JM, O'Higgins F, Buckland-Wright JC, Blake DR. A neurogenic mechanism for symmetrical arthritis. [Review] Lancet 1989;2:1128-30.
41. Gronblad M, Konttinnen YT, Korkala O, Liesi P, Hukkanen M, Polak JM. Neuropeptides in synovium of patients with rheumatoid arthritis and osteoarthritis. J Rheumatol 1988;15:1807-10.
42. Agro A. Stanisz AM. Are lymphocytes a target for substance P modulation in arthritis? Semin Arthritis Rheum 1992;21:252-8.
43. Rangachari PK, McWade D, Donoff B. Luminal tachykinin receptors on canine tracheal epithelium: functional subtyping. Regul Pept 1987;18:101-8.
44. Lotz M, Carson DA, Vaughan JH. Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. Science 1987;235:893-5.
45. Joyce TJ, Yood RA, Carraway RE. Quantitation of substance-P and its metabolites in plasma and synovial fluid from patients with arthritis. J Clin Endocrinol Metab 1993;77:632-7.
46. Mapp PI, Walsh DA, Kidd BL, Cruwys SC, Blake DR. Localization of the enzym neutral endopeptidase to the human synovium. J Rheumatol 1992;19:1838-44.
47. Lam FY, Ferrell WR. Capsaicin suppresses substance-P-induced joint inflammation in the rat. Neurosci Lett 1989;105:155-8.
48. Scott DT, Lam FY, Ferrel WR. Acute inflammation enhances substance P-induced plasma protein extravasation in the rat knee joint. Regul Pept 1992;39:227-35.
49. Mapp PI, Walsh DA, Cruwys SC, Kidd BL, Blake DR. Receptors to the neuropeptide substance P in human synovium. Br J Rheumatol 1990;29 Suppl 1:44.
50. Walsh DA, Mapp PI, Wharton J, Rutherford RAD, Kidd BL, Revell PA, Balke DR, Polak JM. Localisation and characterisation of substance P binding to human synovial tissue in rheumatoid arthritis. Ann Rheum Dis 1992;51:313-7.

CHAPTER 8

SUBSTANCE P RECEPTOR IMAGING

8.1 Substance P receptor scintigraphy: initial studies in rats

8.2 Visualization of the thymus by substance P receptor scintigraphy in man

CHAPTER 8.1

SUBSTANCE P RECEPTOR SCINTIGRAPHY:

INITIAL STUDIES IN RATS

Abstract

We have evaluated the potential usefulness of a new radiolabelled substance P analogue, [^{111}In-DTPA-Arg^1]-substance P, as a radiopharmaceutical for the in vivo detection of substance P receptor positive immunological disorders and tumours. Substance P, [3-(p-hydroxyphenyl)propionyl-Arg^1]-substance P (Bolton-Hunter-substance P, BH-substance P), and [DTPA-Arg^1]-substance P, inhibited dose-dependently the binding of [^{125}I]-BH-substance P to the substance P receptors in rat brain cortex membranes with IC_{50} values of 0.2, 2 and 4 nM, respectively. In an autoradiographic displacement study of the submandibular gland with [^{125}I]-BH-substance P as radiopharmaceutical, an IC_{50} of 2.7 nM was found for [DTPA-Arg^1]-substance P. In vivo metabolism of the radiopharmaceutical in the rat revealed a renal clearance of 50% of the injected radioactive dose in 30 minutes, and a rapid enzymatic degradation of the radiopharmaceutical, resulting in an effective half-life in blood of approximately 3 minutes. Four and 24 hours after the injection of [^{111}In-DTPA-Arg^1]-substance P, uptake in and specific binding to substance P receptor positive organs and tumours were found, with a (substance P receptor positive) target to background optimum at 24 hours. Tissue distribution and ex vivo autoradiographic studies in rats, with and without pretreatment with the selective non-peptide neurokinin 1 receptor antagonist CP96,345, showed uptake and specific binding of radioactivity in isolated tumours, and in the submandibular and parotid glands. Visualization of normal substance P receptor positive tissues such as the salivary glands by gamma camera scintigraphy, after administration of [^{111}In-DTPA-Arg^1]-substance P, was demonstrated in untreated rats. Pathological substance P receptor positive processes were visualized in rats bearing the transplantable pancreatic tumour CA20948, and in rats with adjuvant arthritis, induced by injection of a homogenate of *Mycobacterium tuberculosis*. We conclude that [^{111}In-DTPA-Arg^1]-substance P can be used successfully to visualize substance P receptor positive processes in vivo by substance P receptor scintigraphy.

Introduction

Tachykinins are a family of peptides which share a common C terminal amino acid sequence, -Phe-X-Gly-Leu-Met-NH_2, where X represents either

Phe, Ile, or Val (1). The mammalian tachykinins include substance P, neurokinin A, neurokinin B, and two N terminally extended forms of neurokinin A, i.e. neuropeptide K and neuropeptide Y. Currently accepted tachykinin receptor nomenclature defines three homologous receptor types: (a) the neurokinin 1 receptor, preferring substance P, (b) the neurokinin 2 receptor, preferring neurokinin A, and (c) the neurokinin 3 receptor, preferring neurokinin B. The tachykinin receptors have a wide tissue distribution, and interaction with their ligands is associated with diverse responses, such as sensory neurotransmission, immunological responses, both contraction and endothelium dependent relaxation of vascular smooth muscle, nociception, histamine release, plasma extravasation, inflammation, and, potentially, nerve regeneration and wound healing (1,2).

The involvement of substance P in the carcinoid syndrome (3) and vascular (migraine) headache (4) has been suggested. In rats with adjuvant arthritis the synovium was found positive for high affinity substance P receptors (5). This has also been described in patients with rheumatoid arthritis (6). In addition, very high concentrations of substance P receptors are expressed in the germinal centre of lymph nodes (7), and on a human astrocytoma cell line (8). Large numbers of high affinity substance P receptors are found in surgical specimens obtained from patients with inflammatory bowel disease. The substance P binding neurokinin 1 receptor is expressed by arterioles and venules located in the submucosa, muscularis mucosa, external longitudinal muscle, and serosa (7,9).

In the present study several animal models were used to evaluate the potential usefulness of [^{111}In-DTPA-Arg^{1}]-substance P as a radioligand for the in vivo detection of substance P receptor positive tissues. Firstly, the substance P receptor positive submandibular and parotid gland (10-12) were studied in normal rats. As a second model, rats bearing the substance P receptor positive transplantable rat pancreatic tumour CA20948 were investigated. Thirdly, the possible accumulation [^{111}In-DTPA-Arg^{1}]-substance P was studied in the joints of rats with adjuvant arthritis, induced by *Mycobacterium tuberculosis* (13). The specific non-peptide neurokinin 1 receptor antagonist CP96,345 (14) was used in these models in order to block specific binding of [^{111}In-DTPA-Arg^{1}]-substance P to substance P receptors.

Materials and methods

Radiopharmaceuticals

[3-(p-hydroxyphenyl)propionyl-Arg^1]-substance P (Bolton-Hunter-substance P, BH-substance P), [DTPA-Arg^1]-substance P, the substance P antagonist sendide (15) and [DTPA-Tyr^1]-sendide were purchased from Sanbio (Uden, The Netherlands). The amino acid sequences are shown in Figure 1. Amino acid analysis yielded a peptide content of more than 95%. The identity of the peptides was confirmed by amino acid analysis and fast atom bombardment mass spectrometry. $^{111}InCl_3$ (DRN 4901, 370 MBq/ml in HCl, pH 1.5-1.9) was obtained from Mallinckrodt (Petten, the Netherlands). The radiolabelling of [DTPA-Arg^1]-substance P with ^{111}In to a specific activity of 150 MBq ^{111}In per μg [DTPA-Arg^1]-substance P, and consecutive quality control by instant thin layer chromatography (ITLC, Silica gel) and SEP-PAK C_{18} reversed phase extraction were performed as described earlier (16). High performance liquid chromatography (HPLC) was performed with a Waters 600E multisolvent delivery system, connected to a μBondapak C_{18} reversed phase column (300x3.9 mm, particle size 10 μm). Elution was carried out at a flow of 1.5 ml per minute with a linear gradient of 10 to 50% acetonitrile in 0.1% trifluoroacetic acid in 30 minutes. The latter composition was kept constant for another 5 minutes. The radiochemical purity of the radiolabelled substance P analogues was greater than 98%. Although it is not excluded that additional groups of the peptide participate in ^{111}In complexation, the labelled product is referred to as [^{111}In-DTPA-Arg^1]-substance P. [^{115}In-DTPA-Arg^1]-substance P was prepared by mixing [DTPA-Arg^1]-substance P (stock concentration of 10^{-4} M) with a 80-fold molar excess of $^{115}InCl_3$ (Aldrich, Axel, the Netherlands) in 0.05 M acetic acid, as described earlier (17). The binding characteristics of [DTPA-Arg^1]-substance P and [^{115}In-DTPA-Arg^1]-substance P to the substance P receptor were examined. [^{125}I]-BH-substance P was purchased from Amersham (United Kingdom) and also prepared in our laboratory essentially as described earlier (18). The radioiodination was carried out by adding 105 μg BH-substance P in 150 μl 0.05 M acetic acid and 8 μg chloramine T in 100 μl 0.05 M phosphate buffer (pH 7.5) to 185 MBq (50 μl) $Na^{125}I$ (specific activity 0.62 TBq ^{125}I/mg, Amersham, United Kingdom). The mixture was vortexed for 1 minute. The radioiodination was stopped by adding 1 ml 10% human serum albumin (Merieux, Lyon, France). After vortexing for 30 seconds, 2 ml 5 mM ammonium acetate was added.

Substance P
Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met

[^{111}In-DTPA-Arg^{1}]-substance P
^{111}In - DTPA - Arg - Pro - Lys - Pro - Gln - Gln - Phe - Phe - Gly - Leu - Met

Sendide
Tyr - D-Phe - Phe - D-His - Leu - Met

[^{111}In-DTPA-Tyr^{1}]-Sendide
^{111}In - DTPA - Tyr - D-Phe - Phe - D-His - Leu - Met

Figure 1. Sequences of synthetic substance P, [^{111}In-DTPA-Arg ^{1}]-substance P, sendide and [^{111}In-DTPA-Tyr^{1}]-sendide.

Purification was performed using a SEP-PAK C_{18} reversed phase extraction cartridge (Waters Associates, Milford, Massachusetts, USA), which was washed successively with 5 ml 70% ethanol, 5 ml isopropanol and 5 ml distilled water. After application of the sample, the SEP-PAK C_{18} cartridge was washed with 5 ml distilled water and 5 ml 0.5 M acetic acid, and radioiodinated BH-substance P was eluted with 1 ml 96% ethanol. The solvent was evaporated at 25°C under a gentle stream of nitrogen. HPLC of [^{125}I]-BH-substance P was performed on an identical μBondapak C_{18} reversed phase column as mentioned above, and elution was carried out at a flow of 1 ml per minute with a linear gradient of 40 to 70% methanol in 0.05 mM acetate (pH 5.5) in 50 minutes. The latter composition was kept constant for another 5 minutes. The in vitro binding and autoradiography studies were performed with HPLC purified monoiodinated [^{125}I]-BH-substance P.

Reagents
The selective non-peptide neurokinin 1 receptor antagonist CP96,345 (14) was kindly provided by Pfizer (Capelle aan de IJssel, the Netherlands). Substance P was purchased from Saxon-Biochemicals GMBH (Germany) and luteinizing hormone-releasing hormone (LHRH) and thyrotropin-releasing hormone (TRH) from Hoechst (Germany). Octreotide was obtained from Sandoz (Switzerland) and [DTPA-D-Phe^{1}]-octreotide from Mallinckrodt (the Netherlands). All chemicals used were of the highest purity available.

Substance P receptor binding studies and autoradiography

Receptor binding studies were performed with membrane fractions of the submandibular gland, brain stem, brain cortex and the pancreatic tumour CA20948. The incubation mixture consisted of 40 kcpm of [^{125}I]-BH-substance P, while the remainder of the incubation mixture was identical as described earlier (19). Binding curves and IC_{50} values for displacement of [^{125}I]-BH-substance P binding by unlabelled peptide were calculated using the computer fitting program of Graphpad (ISI software, Philadelphia, Pennsylvania, USA). Receptor autoradiography was carried out as described earlier (20) with some modifications. In short, 10 μm cryostat sections of the tissue samples were mounted onto precleaned gelatin coated microscope slides, and stored at -80°C. To wash out endogenous substance P, sections were preincubated for 15 minutes at room temperature with 50 mM Tris-HCl buffer, pH 7.4. Thereafter, sections were incubated for 1 hour at 20°C in 50 mM Tris-HCl buffer, pH 7.4, containing bovine serum albumin (200 mg/l), bacitracin (40 mg/l), and 5 mM $MnCl_2$ to inhibit endogenous proteases, in the presence of iodinated ligand (400 kcpm/ml, about 0.1 nM). Non-specific binding was determined on parallel sections by adding unlabelled native substance P at a concentration of 1 μM. Incubated sections were washed four times for 5 seconds in ice cold 50 mM Tris-HCl buffer, pH 7.4, and four times for 5 seconds in ice cold distilled water to remove salt. The sections were then dried quickly, and were exposed to Hyperfilm^{TM}3H (Amersham, United Kingdom) for one week in X-ray cassettes without intensifying screens at -80°C. Histology was performed on haematoxylin-azophloxine stained sequential cryosections. Specificity of binding was tested by addition of 1 μM substance P, [DTPA-Arg^1]-substance P, CP96,345, LHRH, TRH and octreotide. Autoradiographic displacement experiments in the submandibular gland were performed as described earlier (21). Ex vivo autoradiography was performed on kidney, parotid and submandibular gland and tumour tissue from male Lewis CA20948 pancreatic tumour bearing rats. For this study rats were injected intravenously with either 3.4 mg CP96,345 per kg body weight or vehicle. After 10 minutes the rats were injected with 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P and exsanguinated 24 hours later.

Tissue distribution and specific binding of substance P in control rats

Male Wistar rats (220-260 g) were injected under ether anaesthesia into the dorsal vein of the penis and/or into a sublingual vein. In order to find the

optimal injected mass of [DTPA-Arg^1]-substance P, groups of three or four rats were injected with 0.1, 1 or 5 μg [DTPA-Arg^1]-substance P labelled with 3 MBq ^{111}In. Parallel groups of three or four rats were injected similarly 10 minutes after intravenous administration of 3.4 mg CP96,345 per kg body weight in 0.05 M acetic acid in 154 mM NaCl, as described by Bertrand et al. (22). Specific binding was defined as the difference in tissue uptake of radioactivity between untreated (total binding) and CP96,345-treated (non-specific binding) rats, and was expressed as percentages of the injected radioactivity per gram tissue, as described previously (17). Twenty-four hours after administration of [^{111}In-DTPA-Arg^1]-substance P the rats were exsanguinated. The following organs and tissues were isolated and subsequently analyzed for radioactivity content: blood, bronchi, lungs, thymus, pancreas, spleen, adrenals, kidneys, aorta, esophagus, jejunum, colon, thigh (soft tissue), liver, parotid and submandibular glands, neck lymph nodes, anterior pituitary gland, brain cortex and striatum. The tissue distribution and metabolism of the ^{111}In labelled substance P analogues in vivo were also studied by gamma camera scintigraphy (23).

Metabolism

Male Wistar rats (220-280 g) were sacrificed at 15 minutes, 1 hour, or 24 hours after administration of the radiolabelled substance P analogue. Blood was collected in ethylenediaminetetraacetic acid (EDTA) containing tubes and immediately cooled on ice. Since substance P is readily metabolized by various enzymes in blood (24,25) samples were immediately centrifuged at 0°C and plasma components were separated by SEP-PAK C_{18} chromatography. Using the separation technique described above, [^{111}In-DTPA-Arg^1]-substance P binds to the SEP-PAK C_{18} stationary phase and is only eluted with ethanol, while ^{111}In-DTPA is not retained on SEP-PAK C_{18} columns. The radioactivity in plasma and urine samples, which was eluted with ethanol from the SEP-PAK C_{18} column, is termed peptide bound radioactivity but was not further characterized.

Substance P receptor scintigraphy in arthritic and tumour bearing rats

Adjuvant arthritis was induced in female rats by injection of a homogenate of *Mycobacterium tuberculosis*, as described earlier (13). Seventeen days after inoculation the rats had developed an adjuvant reactive arthritis, mostly located in the hind leg joints, and a granuloma at the site of inoculation, as was confirmed by light microscopy. These rats were injected with either 37

MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P or 37 MBq (0.5 μg) [^{111}In-DTPA-D-Phe^1]-octreotide (Octreoscan, Mallinckrodt, Petten, The Netherlands), since granuloma can be visualized by octreotide scintigraphy (26). Imaging was performed 24 hours after injection of the radiopharmaceutical. In a second imaging study two groups of four male Lewis rats, bearing the transplantable rat pancreatic tumour CA20948, were used. One group was treated intravenously with 3.4 mg CP96,345 per kg body weight 10 minutes prior to the administration of [^{111}In-DTPA-Arg^1]-substance P, in order to block specific receptor binding of the latter. The other group was similarly treated with vehicle prior to the radiopharmaceutical.

Data analysis

The results are expressed as the mean with standard deviation (SD). One-way analysis of variance (ANOVA), was used for statistical analysis. Means were compared using Bonferroni or Newman-Keuls test (27). Tissue binding values were evaluated using Student's t-test. A $p<0.05$ was considered statistically significant.

Results

The efficiency of labelling of BH-substance P with ^{125}I was 30-35%. Purification of the iodination mixture using the SEP-PAK C_{18} reversed phase cartridge resulted in the elution of mainly radioiodine in the water fraction and peptide bound radioiodine in the ethanol fraction, as revealed by HPLC. Figure 2 shows a typical HPLC analysis of peptide(s) eluted in the SEP-PAK C_{18} ethanol fraction, indicating a radiochemical purity of more than 95% of monoradioiodinated BH-substance P. Simultaneous measurement of UV absorbance at 254 nm and radioactivity shows a clear separation between unlabelled BH-substance P, mono- and diiodinated [^{125}I]-BH-substance P with retention volumes of 37.0, 35.5 and 45.0 ml, respectively, using the methanol gradient. The retention volume of the commercially available [^{125}I]-BH-substance P (specific activity approximately 2000 Ci/mmol, Amersham, United Kingdom) was identical with our monoiodinated [^{125}I]-BH-substance P (data not shown). Since our HPLC purified monoiodinated [^{125}I]-BH-substance P is carrier free, its specific activity will also reach this value of approximately 2000 Ci/mmol for carrier free monoiodinated ^{125}I labelled peptides.

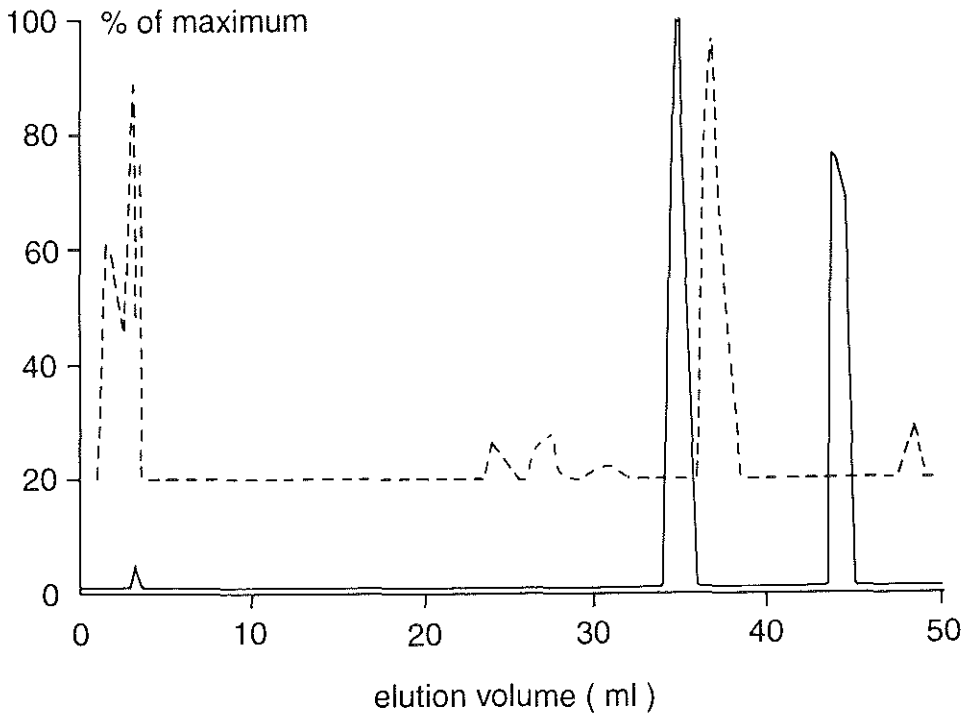

Figure 2. High performance liquid chromatography (HPLC) elution pattern of the ethanol fraction after SEP-PAK C_{18} purification of the BH-substance P radioiodination mixture. Non-radioiodinated BH-substance P (broken line) is measured by UV absorbance (λ = 254 nm), with a retention volume of 37.0 ml. Mono- and diiodinated [^{125}I]-BH-substance P (solid line) are detected by gamma detection at 35.5 and 45.0 ml, respectively.

An over 98% efficiency of labelling of the DTPA conjugated peptides was assured within 15 minutes, as revealed by ITLC and HPLC, when a five- to tenfold molar excess of peptide over ^{111}In was used. Therefore, a specific activity of over 150 MBq ^{111}In per μg [DTPA-Arg^1]-substance P or [DTPA-Tyr^1]-sendide could be achieved. [^{111}In-DTPA-Arg^1]-substance P and [^{111}In-DTPA-Arg^1]-sendide were stable for at least 1 hour after preparation, and for longer than 4 hours if stabilized by addition of the quencher gentisic acid at a final concentration of 2 mg per ml. The retention volumes for [^{111}In-DTPA-Arg^1]-substance P and [DTPA-Arg^1]-substance P with the acetonitrile gradient were 31 and 34 ml, respectively (Figure 3).

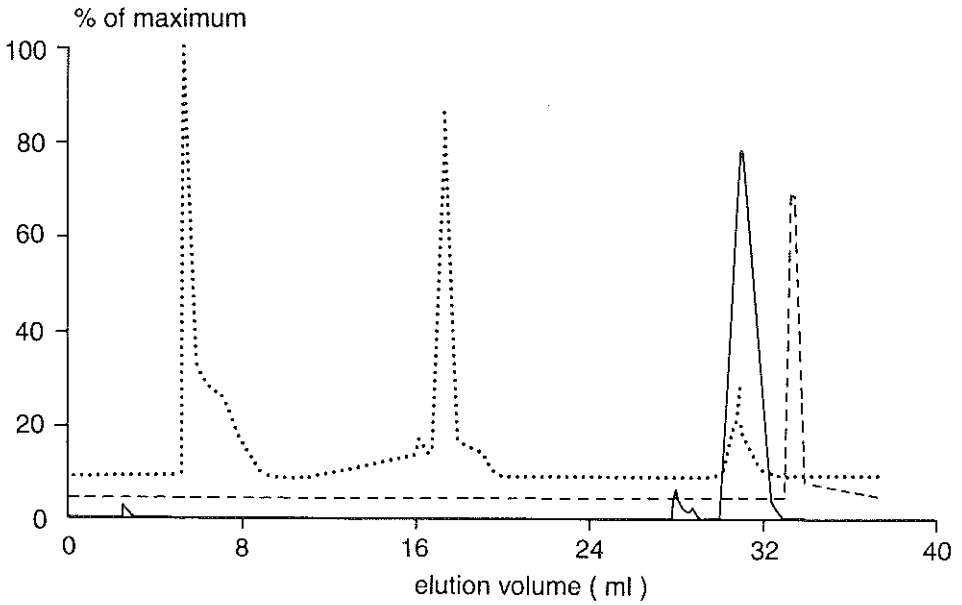

Figure 3. High performance liquid chromatography elution pattern of [^{111}In-DTPA-Arg^1]-substance P (solid line), measured by gamma detection, and [DTPA-Arg^1]-substance P (broken line), measured by UV absorbance (λ = 254 nm) with a 10 to 50% acetonitrile elution gradient. The dotted line represents the HPLC elution pattern of the ethanol fraction of SEP-PAK C_{18} extract of serum collected 15 minutes after injection of the radiopharmaceutical.

Studies of the binding of [^{125}I]-BH-substance P to the substance P receptors in rat parotid gland membranes in the presence of increasing concentrations of substance P, [DTPA-Arg^1]-substance P and [^{115}In-DTPA-Arg^1]-substance P revealed IC_{50} values of 1, 35 and 31 nM, respectively. In brain stem membranes IC_{50} values for substance P and [DTPA-Arg^1]-substance P were 0.5 and 5 nM, respectively, while for sendide and [DTPA-Tyr^1]-sendide IC_{50} values were found in the micromolar range. In another study with membrane fractions of the CA20948 tumour, the binding of [^{125}I]-BH-substance P was inhibited in the presence of 1 μM of substance P, [DTPA-Arg^1]-substance P or CP96,345,respectively, to 67±6, 66±6 and 66±0% of the control value in the absence of competitive ligand. In a study with rat brain cortex membranes IC_{50} values of 0.2, 4 and 2 nM were found for substance P, [DTPA-Arg^1]-substance P and BH-substance P, respectively.

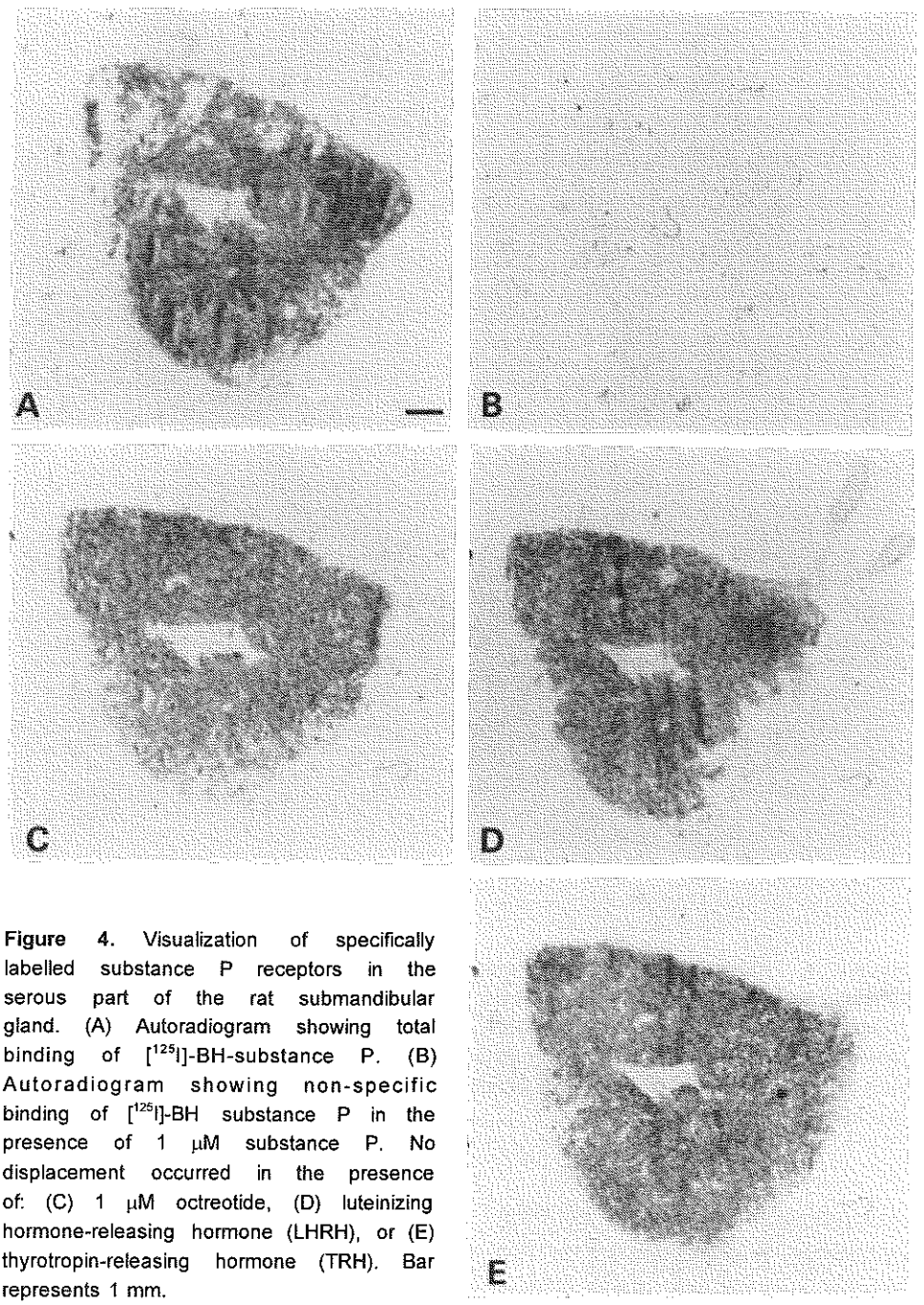

Figure 4. Visualization of specifically labelled substance P receptors in the serous part of the rat submandibular gland. (A) Autoradiogram showing total binding of [^{125}I]-BH-substance P. (B) Autoradiogram showing non-specific binding of [^{125}I]-BH substance P in the presence of 1 μM substance P. No displacement occurred in the presence of: (C) 1 μM octreotide, (D) luteinizing hormone-releasing hormone (LHRH), or (E) thyrotropin-releasing hormone (TRH). Bar represents 1 mm.

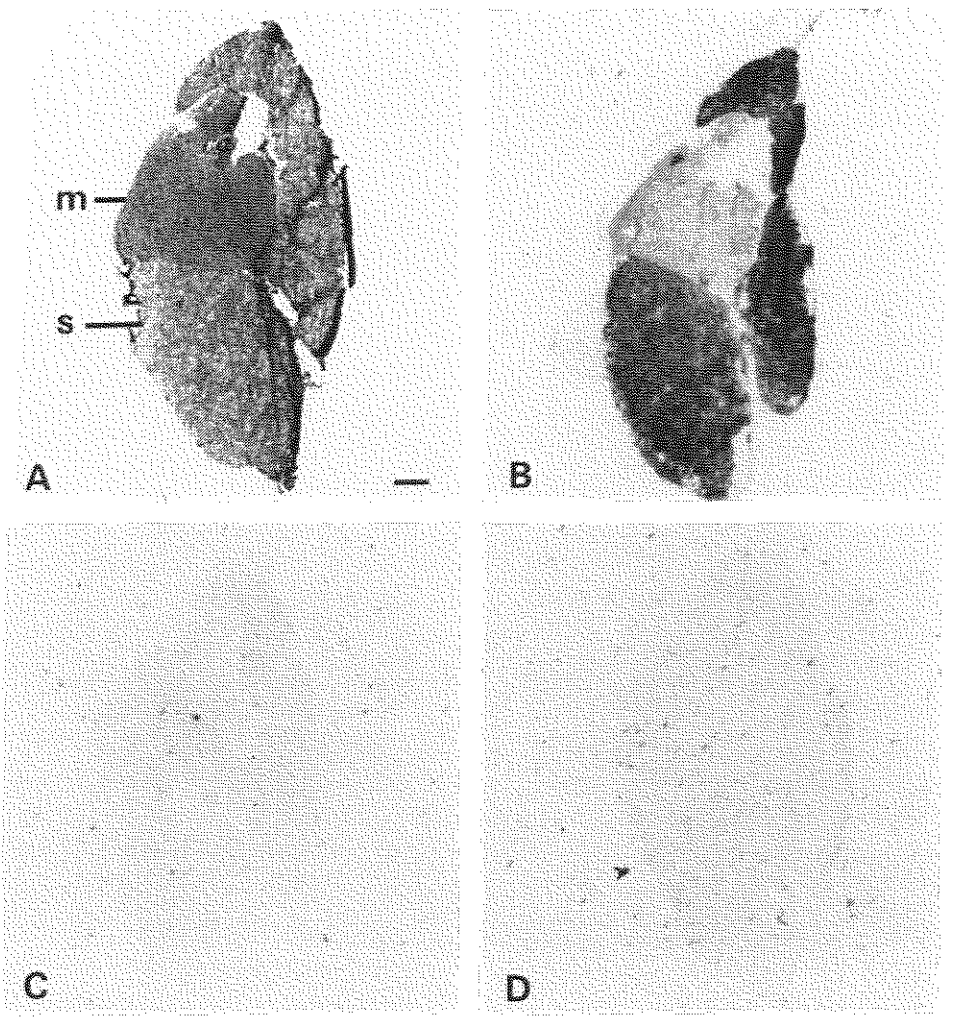

Figure 5. Visualization of the distribution of substance P receptors in rat submandibular gland. (A) Haematoxylin-azophloxine stained section. (B) Autoradiogram showing total binding of [^{125}I]-BH-substance P. (C) Autoradiogram showing non-specific binding of [^{125}I-BH-substance P in the presence of 1 μM substance P. (D) Autoradiogram showing non-specific binding of [^{125}I]-BH-substance P in the presence of 1 μm CP96,345. m = mucous, s = serous

Autoradiography of submandibular gland slices incubated with [^{125}I]-BH-substance P demonstrated a high density of substance P receptors in the serous part of the gland (Figure 4), which contrasted with the lack of substance P receptors in the mucous part (Figure 4 and Figure 5A-D). In the

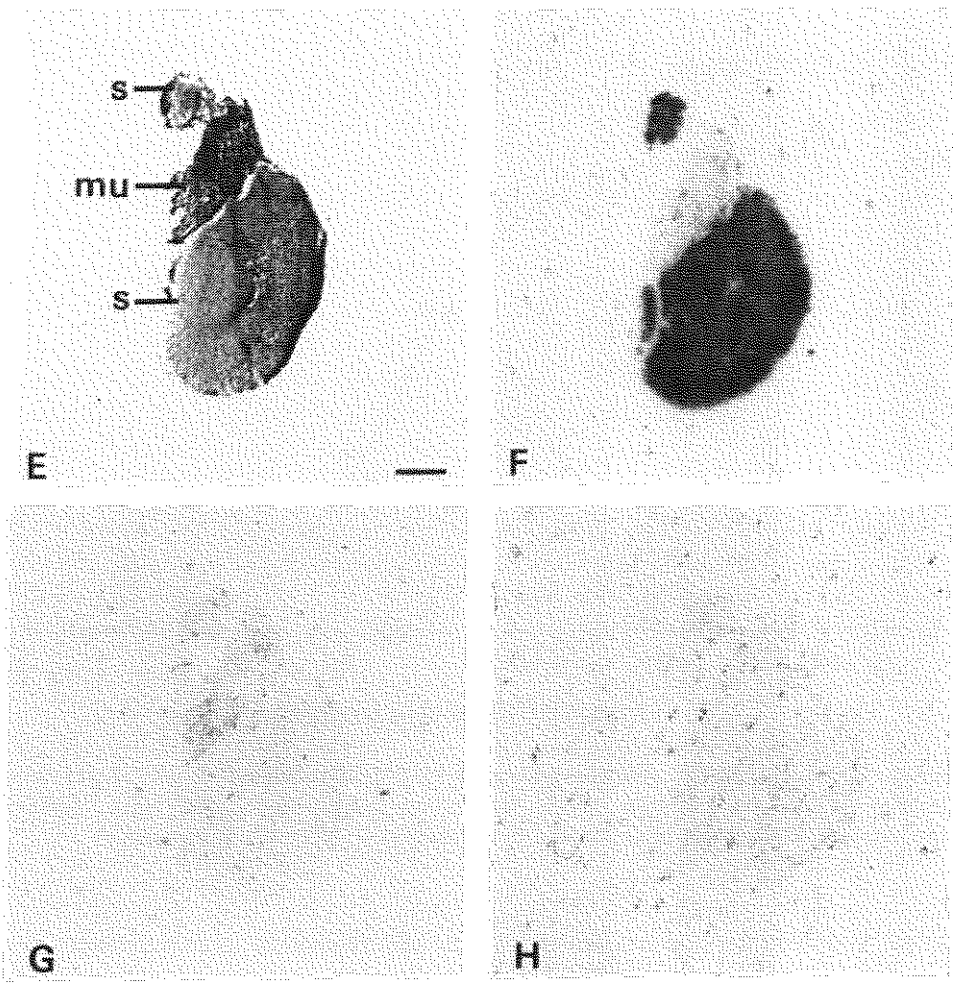

Figure 5. Visualization of the distribution of substance P receptors in rat parotid gland. (E) Haematoxylin-azophloxine stained section. (F) Autoradiogram showing total binding of [^{125}I]-BH-substance P. (G) Autoradiogram showing non-specific binding of [^{125}I]-BH-substance P in the presence of 1 μM substance P. (H) Autoradiogram showing non-specific binding of [^{125}I]-BH-substance P in the presence of 1 μm CP96,345. m = mucous, s = serous, mu = skeletal muscle

presence of 1 μM substance P the binding of [^{125}I]-BH-substance P was completely inhibited, while in parallel incubations with 1 μM octreotide, LHRH or TRH the binding of radioactivity was not affected. The exorbital lacrimal

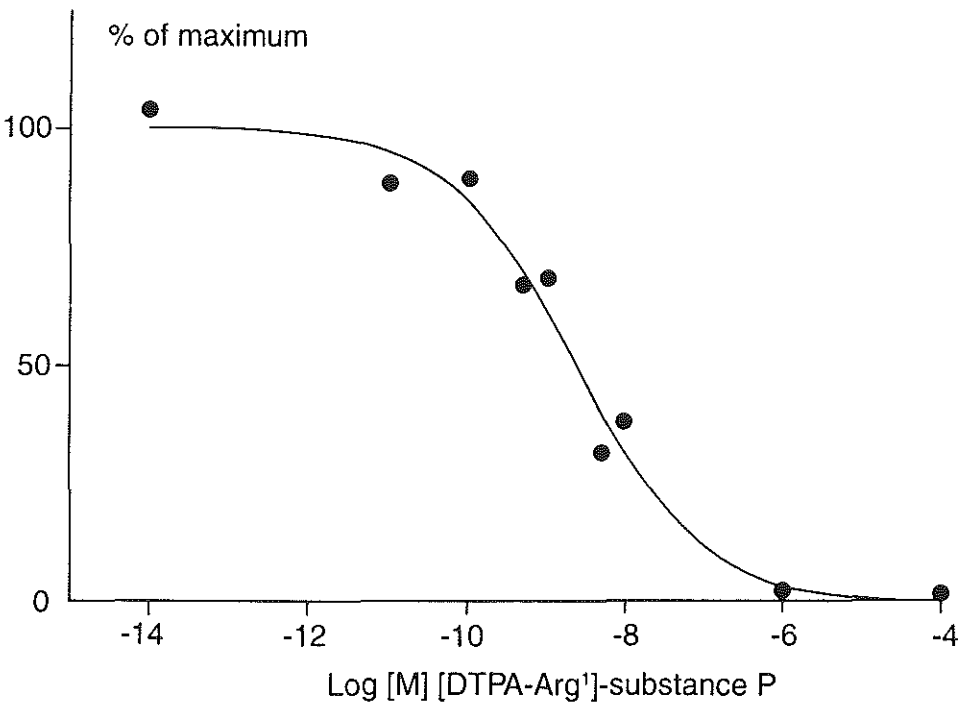

Figure 6. Autoradiographic displacement curve of [^{125}I]-BH-substance P on 10 μm thick sections of rat submandibular gland. Uptake of radioactivity in the tissue sections was assessed at increasing concentrations of the radioligand [DTPA-Arg ^{1}]-substance P.

gland and lymph nodes adjacent to the submandibular gland showed low non-specific binding of [^{125}I]-BH-substance P (data not shown). Figure 5A-H shows the binding of [^{125}I]-BH-substance P to substance P receptors in rat submandibular and parotid gland, respectively, in the absence or presence of 1 μM substance P or 1 μM CP96,345. The serous part of the rat submandibular gland had a high density of high affinity binding sites to the substance P receptor, whereas the mucous part of the gland had a less high density. The rat parotid gland also had a high density of high affinity binding sites to the substance P receptor. Some non-specific binding was present on the skeletal muscle tissue in the parotid gland section.

Figure 6 shows an autoradiographic displacement study using 10 μm slices of the submandibular gland. An IC_{50} value of 2.7 nM was found for

Table 1. Tissue distribution (% of injected dose per gram), 24 hours after intravenous administration of 3 MBq ^{111}In labelled with indicated mass of [DTPA-Arg^1]-substance P in male Wistar rats.

	Administered dose of [^{111}In-DTPA-Arg ^{1}]-substance P					
	0.1 µg (n = 3)		1 µg (n = 3)		5 µg (n = 4)	
Tissue	Mean	SD	Mean	SD	Mean	SD
Blood	0.0062	0.0009	0.0055	0.002	0.0054	0.0005
Lungs	0.022	0.004	0.020	0.004	0.020	0.003
Bronchi	0.068	0.011	0.089	0.020	0.069	0.011
Thymus	0.049	0.004	0.088	0.015	0.048	0.011
Pancreas	0.019	0.004	0.023	0.005	0.022	0.006
Spleen	0.31	0.07	0.32	0.11	0.25	0.06
Adrenals	0.029	0.006	0.033	0.001	0.055	0.013
Kidneys	1.1	0.1	1.9	0.3	2.9	1.1
Aorta	0.023	0.008	0.018	0.008	0.044	0.009
Esophagus	0.049	0.009	0.069	0.020	0.030	0.005
Jejunum	1.1	0.11	1.4	0.2	1.0	0.21
Colon	0.24	0.03	0.22	0.04	0.33	0.10
Soft tissue	0.011	0.002	0.019	0.003	0.015	0.003
Liver	0.18	0.03	0.24	0.02	0.17	0.04
Submandibular gland	0.65	0.07	1.1	0.0	1.3	0.2
Parotid gland	0.16	0.02	0.29	0.05	0.32	0.08
Pituitary gland	0.020	0.001	0.041	0.008	0.015	0.004
Brain cortex	<0.001		<0.001		<0.001	
Striatum	<0.001		<0.001		<0.001	

SD = standard deviation

[DTPA-Arg^1]-substance P.

The target to background ratio increased between 4 and 24 hours, as measured by the uptake of radioactivity in isolated substance P receptor positive tissues (salivary glands = target) compared with the radioactivity in blood (= background). This calculated ratio increased from 23±11 at 4 hours up to 200±16 at 24 hours after the administration of the radiopharmaceutical.

Table 1 and Table 2 show the radioactivity measured in the isolated tissues, 24 hours after injection of 0.1, 1 and 5 µg of [DTPA-Arg^1]-substance P,

Table 2. Specific binding (% of injected dose per gram) in tissues of male Wistar rats, 24 hours after intravenous administration of 3 MBq ^{111}In labelled with indicated mass of [DTPA-Arg^1]-substance P.

	Administered dose of [^{111}In-DTPA-Arg ^{1}]-substance P					
	0.1 μg (n = 3)		1 μg (n = 3)		5 μg (n = 4)	
Tissue	Mean	SD	Mean	SD	Mean	SD
Bronchi	0.044 *	0.011	0.047 *	0.020	0.048 *	0.011
Thymus	0.029 *	0.004	0.058 *	0.015	0.000	0.011
Spleen	0.19 *	0.07	0.27 *	0.04	0.03	0.06
Esophagus	0.029 *	0.009	0.063 *	0.020	0.002	0.005
Jejunum	0.86 *	0.11	0.60 *	0.25	0.0	0.2
Colon	0.17 *	0.03	0.062	0.037	0.0	0.1
Submandibular gland	0.52 *	0.07	0.83 *	0.04	0.77 *	0.15
Parotid gland	0.15 *	0.04	0.21 *	0.08	0.16	0.09
Pituitary gland	0.008 *	0.004	0.033 *	0.008	0.004	0.004

SD = standard deviation

* specific binding different from zero, $p<0.05$

labelled with a constant amount of ^{111}In (3 MBq). Table 1 represents the total binding, and Table 2 the specific binding of [DTPA-Arg^1]-substance P. The high concentration of radioactivity in the kidneys after injection of [^{111}In-DTPA-Arg^1]-substance P was evident, since renal excretion is the predominant route for clearance of [^{111}In-DTPA-Arg^1]-substance P. About 50% of radioactivity was excreted via the kidneys in the first 30 minutes after injection of [^{111}In-DTPA-Arg^1]- substance P. Most of the tissues mentioned in Table 1 and Table 2 were selected as potential targets because of their substance P receptor content, while blood and soft tissue were selected as substance P receptor negative background. Background values were low and were comparable with the values found after the administration of [^{111}In-DTPA-D-Phe^1]-octreotide (23). The uptake of radioactivity in the substance P receptor positive colon, jejunum, and submandibular and parotid glands was also similar to the uptake found in somatostatin receptor positive organs with [^{111}In-DTPA-D-Phe^1]-octreotide scintigraphy (23). However, the uptake of radioactivity in substance P receptor positive adrenals, pituitary gland, aorta, esophagus and lungs was low. No significant uptake nor specific binding of

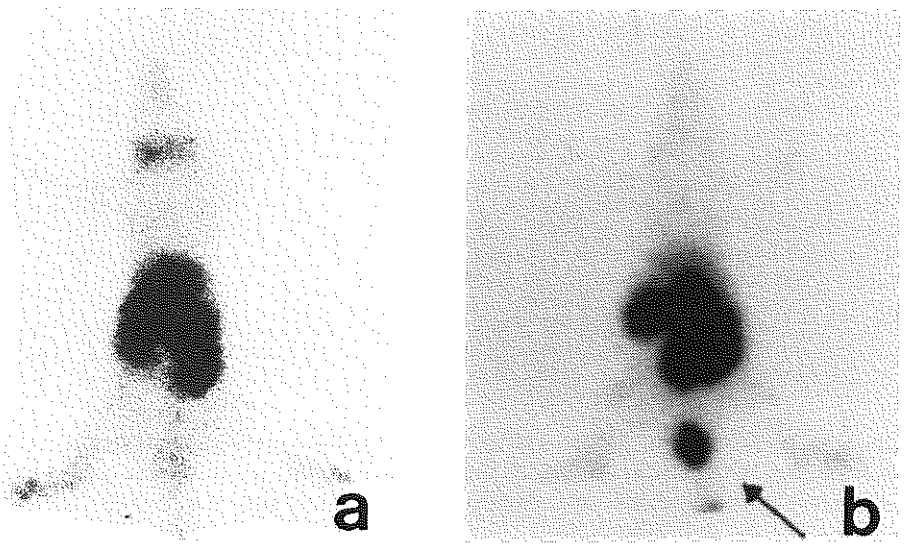

Figure 7. Substance P analogue scintigraphy in rats with adjuvant arthritis, 24 hours after injection of 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P (A) or 37 MBq (0.5 μg) [^{111}In-DTPA-D-Phe^1]-octreotide (B) (posterior view). The arrow indicates the site of inoculation.

[^{111}In-DTPA-Arg^1]-substance P was found in the brain cortex and striatum, suggesting that [^{111}In-DTPA-Arg^1]-substance P was unable to pass the blood-brain barrier. In most tissues total and specific binding were optimal with the intermediate mass (1 μg) of the radiopharmaceutical administered. In CA20948 tumour bearing male Lewis rats total binding amounted to 0.13±0.01% of the injected dose per gram tumour tissue, and specific binding to 0.07±0.01% of the injected dose per gram tumour tissue.

SEP-PAK C_{18} columns were used for analysis of peptide bound radioactivity in blood and urine of rats, 15 minutes, 1 hour and 24 hours after the injection of [^{111}In-DTPA-Arg^1]-substance P. The percentage peptide bound radioactivity in blood was 10±2, 4±1 and 0.5±0.1, respectively. HPLC analysis of the ethanol fractions of the SEP-PAK C_{18} extractions of these blood samples showed that less than 25, 15 and 5%, respectively, of this peptide bound ^{111}In was intact [^{111}In-DTPA-Arg^1]-substance P. The subsequent half-life of the intact radiopharmaceutical in the first 15 minutes after the injection

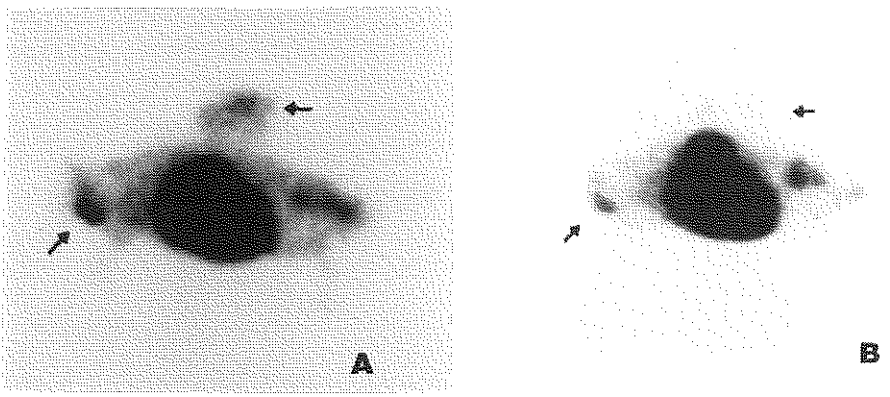

Figure 8. Substance P analogue scintigraphy in CA20948 tumour bearing rats, 24 hours after injection of 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P (lateral view). (A) No pretreatment with CP96,345. (B) Pretreatment with CP96,345. The arrows indicate the salivary gland and the tumour, located at the back of the rat.

approximately 3 minutes. This is illustrated for the 15 minutes serum sample in Figure 3. The nature of the metabolites of [^{111}In-DTPA-Arg^1]-substance P was not further investigated.

The percentage of peptide bound ^{111}In in urine samples collected 1 hour and 24 hours after injection of [^{111}In-DTPA-Arg^1]-substance P was less than 10% and 5%, respectively, of the total radioactivity. More than 90% of the radioactivity excreted in the urine collected 1 hour after injection of the radiopharmaceutical had a molecular weight of less than 1500 dalton, as shown by PD-10 gel chromatography, i.e. smaller than that of the injected [^{111}In-DTPA-Arg^1]-substance P (data not shown).

Dynamic images during the first 20 minutes after injection of [^{111}In-DTPA-Arg^1]-substance P showed a rapid distribution of radioactivity over the whole body. In the first minutes after injection of [^{111}In-DTPA-Arg^1]-substance P the kidneys were rapidly visualized and excretion of radioactivity in the urine was demonstrated. The dynamic images were comparable to those obtained with [^{111}In-DTPA-D-Phe^1]octreotide (23) and [^{111}In-DTPA-D-Phe^1]-RC160 (17). Digital static images obtained 24 hours after injection of [^{111}In-DTPA-Arg^1]-substance P revealed a whole body retention of less than 7% of the injected

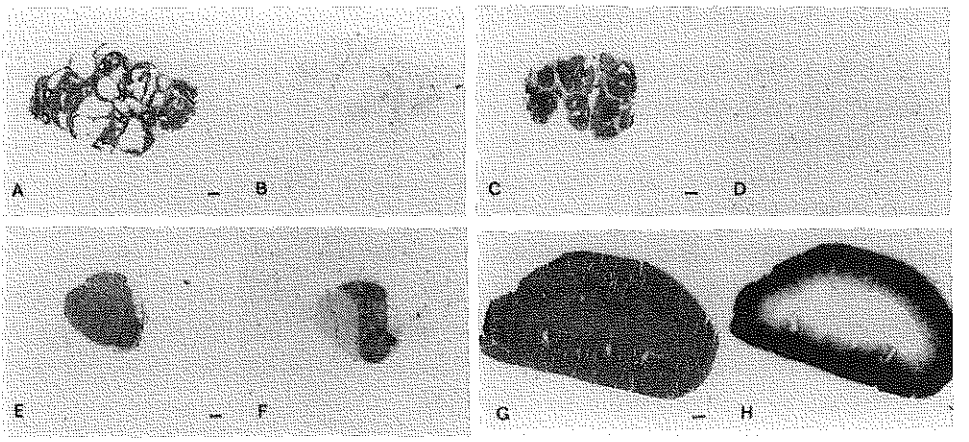

Figure 9. Visualization of substance P receptors in a CA20948 tumour bearing rat by ex vivo autoradiography, after administration of [^{111}In-DTPA-Arg^1]-substance P. (A) Haematoxylin-azophloxine stained section of pancreatic tumour, without CP96,345 pretreatment. (B) Autoradiogram of pancreatic tumour section, without CP96,345 pretreatment. (C) Haematoxylin-azophloxine stained section of pancreatic tumour, with CP96,345 pretreatment. (D) Autoradiogram of pancreatic tumour section, with CP96,345 pretreatment. (E) Haematoxylin-azophloxine stained section of submandibular gland, without CP96,345 pretreatment. (F) Autoradiogram of submandibular gland section, without CP96,345 pretreatment. (G) Haematoxylin-azophloxine stained section of kidney section, without CP96,345 pretreatment. (H) Autoradiogram of kidney section, without CP96,345 pretreatment.

dose, which was predominantly accounted for by the radioactivity in the kidneys, the liver, the salivary glands, the jejunum and the colon (see also Table 1). Figure 7 represents the static analogue images in two rats with adjuvant arthritis of the hind leg joints and an inflammation granuloma at the site of injection of the homogenate of *Mycobacterium tuberculosis*. The images were obtained 24 hours after the injection of 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P (Figure 7A) or 37 MBq (0.5 μg) [^{111}In-DTPA-D-Phe^1]-octreotide (Figure 7B). A clear difference in tissue uptake of these radiopharmaceuticals was seen. The salivary glands and the arthritic hind leg joints had a high uptake of the radiolabelled substance P, while no uptake of [^{111}In-DTPA-D-Phe^1]-octreotide was seen. The granuloma at the site of injection, however, showed a high uptake of the radiolabelled octreotide, but

less uptake of [^{111}In-DTPA-Arg^1]-substance P. A high uptake of radioactivity was seen in the liver and the kidneys in both images.

In Figure 8 the static lateral analogue images of two CA20948 tumour bearing rats are shown, 24 hours after injection of 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P. Accumulation of radioactivity was observed in the tumour, the kidneys and the salivary glands (Figure 8A). Uptake of radioactivity in the tumour, the parotid and submandibular glands was reduced by 50% after pretreatment of the rats with the substance P antagonist CP96,345 (3.4 mg/kg bodyweight) (Figure 8B). The reduction was in agreement with the concentrations of radioactivity that were measured in the isolated organs. The uptake of radioactivity in the kidneys and liver was not affected by pretreatment with CP96,345.

Figure 9 shows the ex vivo autoradiography of the transplantable pancreatic tumour CA20948, with and without pretreatment of the rats with CP96,345 (3.4 mg per kg bodyweight), the submandibular gland, and the kidney, after injection of 37 MBq (1 μg) [^{111}In-DTPA-Arg^1]-substance P. The submandibular gland showed a high uptake of radioactivity in the serous acini, and a lower uptake in the mucous acini, as was also demonstrated in the abovementioned in vitro autoradiographic study. Serous and Paneth cells, localized in the mucous part of the submandibular gland, seem responsible for the uptake of radioactivity in this part of the gland. The autoradiogram of the kidney demonstrated the uptake of radioactivity in the proximal tubuli, which was not affected by pretreatment with CP96,345 (data not shown).

Discussion

[^{125}I]-BH-substance P has been shown to be a specific, high affinity radiopharmaceutical for the neurokinin 1 receptor (substance P receptor). In the present study it was demonstrated that [DTPA-Arg^1]-substance P was a potent competitor for [^{125}I]-BH-substance P binding to substance P receptors in isolated membranes from cerebral cortex, brain stem and salivary glands, as well as in tissue sections of the submandibular gland. These results suggest that [^{111}In-DTPA-Arg^1]-substance P is an attractive radioligand for substance P receptor scintigraphy.

A low uptake of [^{111}In-DTPA-Arg^1]-substance P was seen in blood and soft tissue (substance P receptor negative), while substance P receptor positive organs, such as the colon, the jejunum, the submandibular and parotid glands showed a higher uptake of radioactivity. Uptake of [^{111}In-DTPA-Arg^1]-substance P in the pituitary gland was low, corresponding to the small number of substance P receptors detected in this tissue (28,29). In contrast to the findings of several other groups (30,31) we could not demonstrate specific uptake in the lungs, although we did find uptake and specific binding in the bronchi. The presence of substance P receptors in smooth muscle, arterioles, and venules has been detected by autoradiography with [^{125}I]-BH-substance P as radiopharmaceutical (7,9). However, these structures represent only a minor part of the total mass of the investigated tissues, such as the aorta, bronchi and esophagus. Hence, the total uptake of radioactivity in these tissues is low. The lack of uptake of radioactivity in the brain cortex and the striatum suggests the inability of [DTPA-Arg^1]-substance P and [^{111}In-DTPA-Arg^1]-substance P to penetrate the intact blood-brain barrier. These data suggests that substance P receptor positive brain tumours, such as astrocytoma and glioma, might be visualized by gamma camera scintigraphy only when the blood-brain barrier is affected.

The relatively high non-specific binding of [^{111}In-DTPA-Arg^1]-substance P to substance P receptor positive tissues is in contrast with the high specific uptake of radioactivity in somatostatin receptor positive tissues, as seen in somatostatin receptor scintigraphy in animals. To study the non-specific binding of [^{111}In-DTPA-Arg^1]-substance P we used the substance P antagonist CP96,345, as described by Bertrand et al. (22), since homologous blocking of the substance P receptor is not possible for pharmacological reasons. In rats pretreated with CP96,345 a high level of non-specific tissue uptake of [^{111}In-DTPA-Arg^1]-substance P was still seen. This may partially be due to incomplete blockade of the substance P receptors, since CP96,345 is known to have lower affinity for rat substance P receptors (K_d = 35 nM) than for human substance P receptors (K_d = 0.5 nM) (32-34). Furthermore, little is known about the pharmacokinetics of CP96,345 in rats. Non-specific binding of [^{125}I]-BH-substance P in the presence of CP96,345 was relatively low in the in vitro autoradiography tissue. However, the antagonist was added at a high concentration (1 μM), and it is unknown whether the concentration required to saturate substance P receptors is reached in vivo after administration of 3.4 mg CP96,345 per kg bodyweight. Therefore, our

estimates of the non-specific binding of [^{111}In-DTPA-Arg^1]-substance P in tissues using this dose of CP96,345 may be overestimations of the true values. The total uptake of [^{111}In-DTPA-Arg^1]-substance P in substance P receptor positive tissues was also very low compared with results obtained by somatostatin receptor scintigraphy in somatostatin receptor positive tissues.

The tissue distribution of [^{111}In-DTPA-Arg^1]-substance P was studied after administration of varying masses of [DTPA-Arg^1]-substance P labelled with a constant amount of ^{111}In (3 MBq). In contrast to the general belief that receptor scintigraphy shows an optimal target to background ratio at the lowest possible mass of the radiopharmaceutical with the highest specific radioactivity, we found that the uptake of [^{111}In-DTPA-Arg^1]-substance P in substance P receptor positive tissue was usually optimal at intermediate doses. This phenomenon may also apply to substance P receptor positive immunological disorders, the visualization of which may be enhanced by optimizing the mass of [^{111}In-DTPA-Arg^1]-substance P. We previously reported similar findings on the binding of [^{111}In-DTPA-D-Phe^1]-octreotide to somatostatin receptor positive tissues (35,36). However, the tissue specific bell-shaped function of [^{111}In-DTPA-D-Phe^1]-octreotide uptake versus the injected mass of [DTPA-D-Phe^1]-octreotide was explained in part by the homologous upregulation of the somatostatin receptors. It is unknown if substance P receptors are (up- or down)regulated by substance P, but it has been reported that substance P receptors are downregulated by substance P antagonists (37).

In vivo metabolism of [^{111}In-DTPA-Arg^1]-substance P in the rat revealed a renal clearance of 50% of the injected radioactivity in 30 minutes, and a rapid enzymatic degradation of the radiopharmaceutical, resulting in an effective half-life of the intact radiopharmaceutical in blood of approximately 3 minutes. The presence of the DTPA-group in [^{111}In-DTPA-Arg^1]-substance P does not seem to affect the biological half-life in serum, since similar half-lives have been reported for serum in dogs (2.1 minutes) (24) and humans (1.6 minutes) (3). Less than 10% of the injected dose was found intact in the urine, during the first 30 minutes after injection of the radiopharmaceutical, which is another indication of the rapid degradation of the radiopharmaceutical. These data are in contrast with previously reported findings on [^{111}In-DTPA-D-Phe^1]-octreotide (23) and [^{111}In-DTPA-D-Phe^1]-

RC160 (17) scintigraphy in rats, where a larger fraction of the radiopharmaceutical was excreted intact in the urine. Nevertheless, salivary glands, arthritic joints, and the substance P receptor positive tumour were visualized by gamma camera scintigraphy. These data suggest that the receptor-ligand interactions in vivo must be very rapid, since the concentration of intact radiopharmaceutical in the circulation rapidly diminishes.

Sendide was reported to be a potent and selective neurokinin 1 receptor antagonist (15). In our hands, however, [^{111}In-DTPA-Tyr^1]-sendide did not show selective binding characteristics to the neurokinin 1 receptor, neither in vitro nor in vivo. After intravenous administration of [^{111}In-DTPA-Tyr^1]-sendide in rats a rapid clearance was observed, without specific binding to the substance P receptor positive salivary glands or to any part of the gastrointestinal tract.

The binding characteristics of [^{111}In-DTPA-Arg^1]-substance P to human substance P receptors is currently under investigation. Several studies have reported discrepancies between the potencies of several substance P analogues in different species, and differences of less than 10% in the amino acid sequences of substance P receptors of rats and humans (2,38). Different types of substance P receptors are also reported in rat submandibular gland (39). Before [^{111}In-DTPA-Arg^1]-substance P can be applied in humans, the pharmacological properties of radiolabelled and unlabelled [DTPA-Arg^1]-substance P have to be investigated thoroughly, since substance P is known to exert various unwanted cardiovascular effects. Schaffalitzky et al. (3) and Coiro et al. (40-42) have demonstrated that such side effects do not occur when substance P is infused at low infusion rates (1.5 pmol/kg per minute). However, for the visualization of substance P receptor positive processes, substance P antagonists may be more attractive radiopharmaceuticals than substance P.

We conclude that [^{111}In-DTPA-Arg1]-substance P can be used successfully to visualize substance P receptor positive tissues in vivo by substance P receptor scintigraphy. Further studies in patients with immunological disorders need to be performed, with special attention to disorders that have been proven to be substance P receptor positive.

References

1. Mussap CJ, Gerarghty DP, Burcher E. Tachykinin receptors: a radioligand binding perspective. [Review] J Neurochem 1993;60:1987-2009.
2. Gerard NP, Bao L, Xiao-Ping H, Gerard C. Molecular aspects of the tachykinin receptors. [Review] Regul Pept 1993;43:21-35.
3. Schaffalitzky de Muckadell OB, Aggestrup S, Stentoft P. Flushing and plasma substance P concentration during infusion of synthetic substance P in normal man. Scand J Gastroenterol 1986;21:498-502.
4. Moskowitz MA, Barley PA. The trigeminovascular system and vascular head pain: a role for substance P. In: Jordan CC, Oehme P, editors. Substance P: metabolism and biological actions. London: Taylor and Francis, 1985:153-63.
5. Walsh DA, Salmon M, Wharton J, Mapp PI, Polak JM. Autoradiographic localisation and characterisation of substance P binding sites in rat knees. Regul Pept 1993;46:189-92.
6. Walsh DA, Mapp PI, Wharton J, Rutherford RAD, Kidd BL, Revell PA, Blake DR, Polak JM. Localisation and characterisation of substance P binding to human synovial tissue in rheumatoid arthritis. Ann Rheum Dis 1992;51:313-7.
7. Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EP Jr, Vigna SR, Maggio JE, Kruger L, Mantyh PW. Receptor binding sites for substance P, but not substance K, or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens from patients with ulcerative colitis and Crohn disease. Proc Natl Acad Sci U S A 1988;85:3235-9.
8. Heuillet E, Ménager V, Fardin V, Flamand O, Bock M, Garret C, Crespo A, Fallourd AM, Doble A. Characterization of a human NK1 tachykinin receptor in the astrocytoma cell line U 373 MG. J Neurochem 1993;60:868-76.
9. Mantyh PW, Gates T, Mantyh CR, Maggio JE. Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J Neurosci 1989;9:258-79.
10. Liang T, Cascieri MA. Substance P receptor on parotid cell membranes. J Neurosci 1981;1:1133-41.
11. Buck SH, Burcher E. The rat submaxillary gland contains predominantly P-type tachykinin binding sites. Peptides 1985;6:1079-84.
12. Boyd ND, White CF, Cerpa R, Kaiser ET, Leeman SE. Photoaffinity labeling the substance P receptor using a derivative of substance P containing p-benzophenylalanine. Biochemistry 1991;30:336-42.
13. Holoshitz J, Matitiau A, Cohen IR. Role of the thymus in induction and transfer of vaccination against adjuvant arthritits with a T lymphocyte line in rats. J Clin Invest 1985;75:472-7.
14. Snider RM, Constantine JW, Lowe JA 3d, Longo KP, Lebel WS, Woody HA, Drozda SE, Desai MC, Vinick FJ, Spencer RW, Hess HJ. A potent non peptide antagonist of the substance P (NK1) receptor. Science 1991;251:435-7.
15. Sakurada T, Manome Y, Tan-No K, Sakurada S, Kisara K, Ohba M, Terenius L. A selective and extremely potent antagonist of the neurokinin-1 receptor. Brain Res 1992;593:319-22.

16. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, Lamberts SWJ, Visser TJ, Krenning EP. [^{111}In-DTPA-D-Phe ^{1}]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991;49:1583-91.
17. Breeman WAP, Hofland LJ, van der Pluijm M, van Koetsveld PM, de Jong M, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Visser TJ, Lamberts SWJ, Krenning EP. 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. Eur J Nucl Med 1994;21:328-35.
18. Breeman WAP, Hofland LJ, Bakker WH, Pluijm van der M, van Koetsveld PM, de Jong M, Setyono-Han B, Kwekkeboom DJ, Visser TJ, Lamberts SWJ, Krenning EP. Radioiodinated somatostatin analogue RC-160: preparartion, biological activity, in vivo application in rats and comparison with [^{123}I-Tyr^{3}]octreotide. Eur J Nucl Med 1993;20:1089-94.
19. Reubi JC. New selective radioligand for one subpopulation of brain somatostatin receptors. Life Sci 1985;36:1829-36.
20. Reubi JC, Waser B, Horisberger U, Halter F, Soroka CJ, Kumar RR, Goldenring JR, Modlin IM. Identification of somatostatin and gastrin receptors on enterochromaffin-like cells from Mastomys gastric tumors. Endocrinology 1992;131:166-72.
21. Reubi JC, Kvols LK, Waser B, Nagorney DM, Heitz PU, Charboneau JW, Reading CC, Moertel C. Detection of somatostatin receptors in surgical and percutaneous needle biopsy samples of carcinoids and islet cell carcinomas. Cancer Res 1990;50:5969-77.
22. Bertrand C, Geppetti P, Baker J, Yamawaki I, Nadel YA. Tachykinins, via NK1 receptor activation, play a relevant role in plasma protein extravasation evoked by allergen challenge in the airways of sentized guinea-pigs. Regul Pept 1993;46:214-6.
23. Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyono-Han B, de Jong M, Kooy PPM, Bruns C, van Hagen PM, Marbach P, Visser TJ, Pless J, Lamberts SWJ. In vivo application of [^{111}In-DTPA-D-Phe^{1}]-octreotide for detection of somatostatin receptor- positive tumors in rats. Life Sci 1991;49:1593-601.
24. Kabemura T, Misawa T, Chijiiwa Y, Nasu T, Nawata H. Substance P, vasoctive intestinal polypeptide, and gastrin catabolism in canine liver and kidney. Dig Dis Sci 1992;37:1661-5.
25. Joyce TJ, Yood RA, Carraway RE. Quantitation of substance P and its metabolites in plasma and synovial fluid from patients with arthritis. J Clin Endocrinol Metab 1993;77:632-7.
26. van Hagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue JA, Hoogsteden HC, Lamberts SWJ. Somatostatin analogue scintigraphy in granulamatous diseases. Eur J Nucl Med 1994;21:497-502.
27. Snedecor GW, Cochran WG. Statistical Methods. 7th ed. Ames: Iowa State University Press, 1980.

28. Larsen PJ, Mikkelsen JD, Mau SE, Særmark T. Binding and internalization of a iodinated substance P analog by cultured anterior pituitary cells. Mol Cell Endocrinol 1989;65:91-101.
29. Larsen PJ, Særmark T, Mau SE. Binding of an iodinated substance P analogue to cultured anterior pituitary prolactin- and luteinizing hormone-containing cells. J Histochem Cytochem 1992;40:487-93.
30. Carstairs JR, Barnes PJ. Autoradiograhic mapping of substance P receptors in lung. Eur J Pharmacol 1986;127:295-6.
31. del Rosario RB, Gildersleeve DL, Mangner TJ, Wieland DM, Shreve PD, Lowe J, Drozda SE, and Snider RM. Synthesis of a new non-peptide substance P analogue for PET studies [abstract]. J Nucl Med 1992;33:860P.
32. Fong TM, Yu H, Strader CD. Molecular basis for the species selectivity of the neurokinin-1 receptor antagonists CP-96,345 and RP67580. J Biol Chem 1992;267:25668-71.
33. Beaujouan JC, Heuillet E, Petitet F, Saffroy M, Torrens Y, Glowinski J. Higher potency of RP 67580, in the mouse and the rat compared with other nonpeptide and peptide tachykinin NK1 antagonists. Br J Pharmacol 1993;108:793-800.
34. Petitet F, Beaujouan JC, Saffroy M, Torrens Y, Fardin V, Glowinski J. NK-1 tachykinin receptor in rat and guinea pig brains: pharmacological and autoradiographical evidence for a species difference. Peptides 1993;14:551-9.
35. Breeman WAP, Kwekkeboom DJ, Kooij PPM, Bakker WH, Visser TJ, Lamberts SWJ, Krenning EP. The unexpected effects of dose and specific activity on tissue distribution of [^{111}In-DTPA-D-Phe^1]-octreotide in rats. Attempts to optimize the target to background ratio. J Nucl Med 1994;35,162P.
36. Kooij PPM, Kwekkeboom DJ, Breeman WAP, Bakker WH, Visser TJ, Lamberts SWJ, Krenning EP. The effects of specific activity on tissue distribution of [^{111}In-DTPA-D-Phe^1]-octreotide in humans. J Nucl Med 1994;35:226P.
37. Inoue A, Takeda R, Fukuyasu T, Nakata Y, Segawa T. Agonist-induced substance P receptor down-regulation in rat central nervous system. Pharm Res 1988;5:795-9.
38. Burbach JPH, Meijer OC. The structure of neuropeptide receptors. [Review] Eur J Pharmacol 1992;227:1-18.
39. Kage R, Leeman SE, Boyd ND. Biochemical characterization of two different forms of the substance P receptor in rat submaxillary gland. J Neurochem 1993;60:347-51.
40. Coiro V, Volpi R, Capretti L, Speroni G, Bocchi R, Caffarri G, Colla R, Rossi G, Chiodera P. Intravenously infused substance P enhances basal and growth hormone (GH) releasing hormone-stimulated GH secretion in normal men. Peptides 1992;13:843-6.
41. Coiro V, Volpi R, Capretti L, Caiazza A, Marcato A, Bocchi R, Colla R, Rossi G, Chiodera P. Luteinizing hormone response to an intravenous infusion of substance P in normal men. Metabolism 1992;41:689-91.
42. Chiodera P, Coiro V. Effects of intravenous infusion of substance P on arginine vasopresin and oxytocin secretion in normal men. Brain Res 1992;569:173-6.

CHAPTER 8.2

VISUALIZATION OF THE THYMUS BY SUBSTANCE P RECEPTOR SCINTIGRAPHY IN MAN

Abstract

Substance P, an eleven amino acid neuropeptide, plays an important role in the modulation of pain transmission through neurokinin 1 and 2 receptors. Substance P and other tachykinins may play a role in the pathogenesis of inflammatory diseases as well. In this study we present our results concerning the metabolism of the substance P analogue [^{111}In-DTPA-Arg^1]-substance P in men, as well as the visualization of the thymus in patients with immune mediated diseases. Twelve selected patients, five with inflammatory bowel disease, one with ophthalmic Graves' disease, one with sarcoidosis, one with Sjögren's syndrome, one with rheumatoid arthritis, two with myasthenia gravis, and one with systemic lupus erythematosus were investigated. During and after intravenous administration of 200 MBq (2.5 μg) [^{111}In-DTPA-Arg^1]-substance P, blood pressure, heart rate, and oxygen saturation were monitored. Radioactivity was measured in blood, urine and faeces during 48 hours after injection. Planar and single photon emission computed tomography (SPECT) images were obtained 4 and 24 hours after injection. After administration of [^{111}In-DTPA-Arg^1]-substance P a transient flush was observed in all patients. Degradation of [^{111}In-DTPA-Arg^1]-substance P started in the first minutes after administration, resulting in a half-life of 10 minutes for the total plasma radioactivity, and of 4 minutes for the intact radiopharmaceutical, as identified with high performance liquid chromatography (HPLC). Urinary excretion amounted in >95% of the radioactivity within 24 hours post injection, and up to 0.05% was found in the faeces up to 60 hours. In all patients uptake of radioactivity was found in the parotid glands, areolae of the mammae (in women), liver, kidneys, and urine bladder. In eight patients a high uptake of [^{111}In-DTPA-Arg^1]-substance P was observed in the thymus. We conclude that, despite its short half-life, [^{111}In-DTPA-Arg^1]-substance P, a new radiopharmaceutical, can be used to visualize the thymus. This may contribute to the investigation of the role of thymus in immune related diseases. Also, inflammatory sites in various diseases could be visualized.

Introduction

Substance P is an eleven amino acid neuropeptide and is known as a powerful member of the family of tachykinins which are characterized by the C terminal sequence -Phe-X-Gly-Leu-Met-NH_2 (1). It has been well established that

substance P plays an important role in modulating pain transmission from peripheral and central primary afferents through neurokinin 1 and 2 receptors.

Substance P and other tachykinins may play a role in the pathogenesis of inflammatory diseases (2-7). Substance P was shown to induce degranulation of mast cells in vitro, regulate chemotaxis, release lysosomal enzymes from macrophages, and regulate expansion of T and B cells and production of immunoglobulins (8-11). The thymus seems to be involved in immune mediated diseases. Lymphofollicular hyperplasia of the thymus has been described in autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Hashimoto's thyroiditis and Sjögren's syndrome (12-15).

Recently, substance P was detected in the outer cortex of the thymus and in the peptidergic neurons that innervate the thymus (16,17). Specific substance P receptors were found in the rat thymus in association with the vessels and the medulla (18). Large numbers of high affinity binding substance P receptors were found in surgical specimens obtained from patients with inflammatory bowel disease. The substance P binding neurokinin 1 receptor was expressed by arterioles and venules located in the submucosa, muscularis mucosa, external longitudinal muscle, and serosa (2).

We developed a scintigraphic technique using an ^{111}In labelled substance P analogue, [^{111}In-DTPA-Arg^1]-substance P, to visualize affected tissues in immune disorders, e.g. the thymus. In this study results are presented concerning the metabolism of ^{111}In labelled [DTPA-Arg^1]-substance P and the scintigraphic detection of the thymus in immune mediated diseases.

Methods

Patients

Twelve selected patients were investigated (with informed consent), five with inflammatory bowel disease, one with ophthalmic Graves' disease, one with sarcoidosis, one with Sjögren's syndrome, one with rheumatoid arthritis, two with myasthenia gravis, and one with systemic lupus erythematosus. The diagnosis was established according to the clinical presentation and histopathological examination of gastrointestinal biopsies in the patients with inflammatory bowel

disease, and by liver biopsy in the patient with sarcoidosis. In rheumatoid arthritis, Sjögren's syndrome and systemic lupus erythematosus the diagnosis was made according to the internationally accepted diagnostic criteria. The patient with Graves' disease had lowered serum thyroid stimulating hormone concentration and elevated serum free thyroxine and specific thyroid stimulating immunoglobulin concentrations. The diagnosis myasthenia gravis was based on the characteristic medical history and findings at physical examination, the presence of antibodies against acetylcholine receptors and positive anticholinesterase tests. Uptake of radioactivity was investigated in six control patients, i.e. three with carcinoid tumours, one with carcinoma of the lung and two with non-Hodgkin's lymphoma.

Radiopharmaceuticals

[DTPA-Arg^1]-substance P was labelled with $^{111}InCl_3$ (370 MBq/ml in HCl, pH 1.5-1.9, Mallinckrodt, Petten, The Netherlands), up to a specific activity of 150 MBq ^{111}In per μg [DTPA-Arg^1]-substance P. Quality control by instant thin layer chromatography (ITLC, Silica-gel) and SEP-PAK C_{18} reversed-phase extraction were performed essentially as described earlier (19,20). HPLC was performed using a Waters 600E multisolvent delivery system, connected to a μBondapak C_{18} reversed-phase column (300x3.9 mm, particle size 10 μm). Elution was carried out at a flow of 1.5 ml per minute with a linear gradient of 10 to 50% acetonitrile in 0.1% trifluoroacetic acid in 30 minutes, and the latter composition was kept constant for another 5 minutes. The radiochemical purity of the radiolabelled substance P analogue was greater than 98%. Although it is not excluded that additional groups of the peptide participate in ^{111}In complexation, the labelled product is referred to as [^{111}In-DTPA-Arg^1]-substance P.

Substance P receptor imaging

In our study 2.5-5.0 μg [^{111}In-DTPA-Arg^1]-substance P (200 MBq) was infused intravenously in 10 minutes. In all patients blood pressure, heart rate and oxygen saturation of the blood were monitored. Planar and SPECT images were obtained with a large field gamma camera equipped with a medium-energy parallel-hole collimator. SPECT analysis was performed with a Wiener filter on original data. The preset counting time for images obtained 24 hours and 48 hours after injection of [^{111}In-DTPA-Arg^1]-substance P was 15 minutes. Planar images were obtained from the head and neck, chest, upper abdomen and lower abdomen. SPECT studies were performed 4 or 24 hours after injection of

the radiopharmaceutical.

Measurement of radioactivity in blood, urine and faeces
Radioactivity in blood, urine and faeces was measured with an LKB-1282-Compugamma system or a GeLi-detector equipped with a multi channel analyzer (Series 40, Canberra). Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and immediately cooled on ice. Since substance P is readily metabolized by various enzymes in blood, samples were immediately centrifuged at 0°C, and plasma was fractionated on SEP-PAK C_{18} columns. Using the separation technique described previously (20), [^{111}In-DTPA-Arg^1]-substance P binds to SEP-PAK C_{18} stationary phase and is only eluted with ethanol, while ^{111}In-DTPA is not retained on SEP-PAK C_{18} columns. The radioactivity in plasma and urine samples, which was eluted with ethanol from the SEP-PAK C_{18} column, is termed peptide bound radioactivity, but was not further characterized. Blood samples were collected directly before and 2, 5, 10, 20, and 40 minutes, and 1, 4, 20 and 48 hours after infusion. Urine was collected from the time of injection in two 3-hour intervals and thereafter in intervals of 6 hours until 48 hours after injection. If feasible, faeces was collected until 72 hours after injection. The chemical status of the radionuclide in blood and urine was analyzed as a function of time using the SEP-PAK C_{18}, HPLC and PD-10 gel filtration techniques as described previously (19,20).

Results

After administration of [^{111}In-DTPA-Arg^1]-substance P a transient flush was observed in all patients. In one patient with Crohn's disease and anaemia, significant hypotension was observed associated with bradycardia. This patient recovered after 30 seconds in Trendelenburg's position, suggesting the presence of a vasovagal collapse.

In Figure 1 the time courses of total and peptide bound radioactivity in plasma are presented until 48 hours after administration. The average plasma radioactivity decreased rapidly after injection of [^{111}In-DTPA-Arg^1]-substance P. Degradation of [^{111}In-DTPA-Arg^1]-substance P started in the first minutes after administration, resulting in a half-life of 10 minutes for total plasma radioactivity and of 4 minutes for the intact radiopharmaceutical, as identified with HPLC.

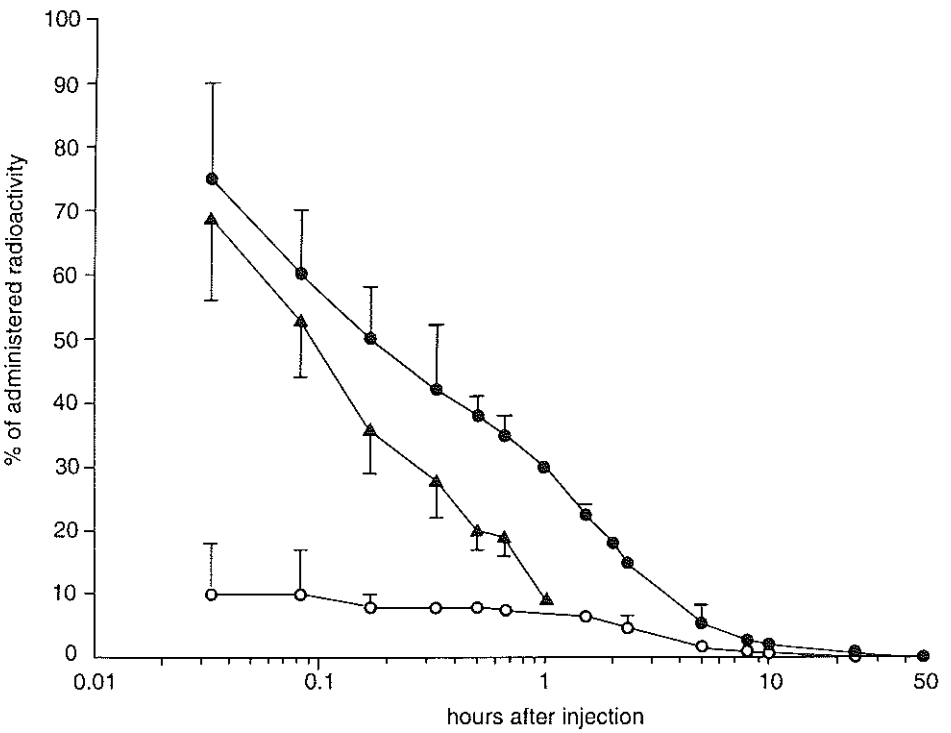

Figure 1. Total plasma (●), peptide bound (▲) and cell bound (○) radioactivity during 48 hours after injection of [^{111}In-DTPA-Arg^1]-substance P.

Renal excretion resulted in the excretion of >95% of the radioactivity in the urine within 24 hours after injection, as shown in Figure 2. Up to 0.05% was found in the faeces until 60 hours after injection.

In all patients uptake of radioactivity was found in the salivary glands (low uptake), liver, kidneys, urine bladder, and in women in the areolae of the mammae 4 hours after injection. The uptake of radioactivity at these sites was supposed to be due to receptor binding (parotid gland and areolae) or metabolism (liver, kidneys and urine bladder) of the ^{111}In labelled substance P analogue. Deposition of radioactivity at other sites was considered to be due to other causes, which may be pathology related. In eight patients a high uptake of [^{111}In-DTPA-Arg^1]-substance P was found in the thymus. The highest uptake of radioactivity in the thymus was observed in the two patients with liver

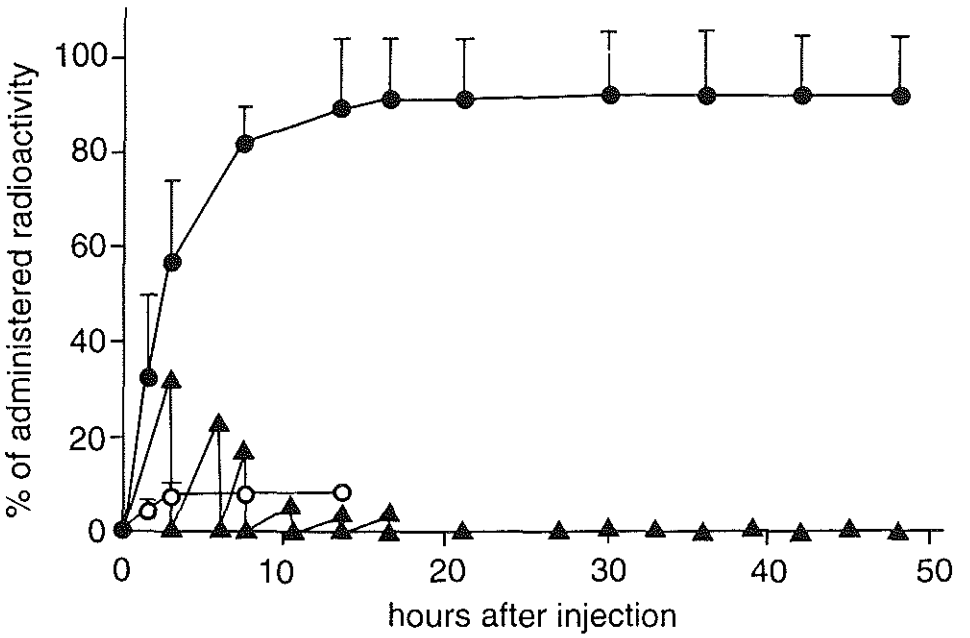

Figure 2. Cumulative urinary excretion pattern of total (●) and peptide bound (○) radioactivity during 48 hours after injection. The radioactivity in the urinary bladder is also shown (▲).

sarcoidosis and Sjögren's syndrome (Figure 3 and Figure 4). CT scanning of the thorax confirmed an enlarged thymus in both patients. In patients with systemic lupus erythematosus, ophthalmic Graves' disease, rheumatoid arthritis and two patients with inflammatory bowel disease the thymus was visualized as well. In one patient with myasthenia gravis the thymus was visualized. In contrast, the other patient with myasthenia gravis, using high dose corticosteroids, was negative at scintigraphy. No uptake of radioactivity in the thymus was observed in the three control patients with either carcinoid tumours, lung carcinoma or non-Hodgkin's lymphoma. In addition to the thymus, in the patient with Sjögren's syndrome, the affected parotid glands showed a high uptake of radioactivity (Figure 5). In the patient with unilateral ophthalmic Graves' disease, high uptake of radioactivity was observed in the eye mostly affected (Figure 6). In three of the five patients with inflammatory bowel disease accumulation of radioactivity was found at the sites of inflammation (data not shown).

Figure 3. Three-dimensional representation of the chest of a patient with Sjögren's syndrome, in whom the thymus is visualized, by ^{111}In labelled substance P analogue scintigraphy (24 hours after injection).

Discussion

Substance P is found in the central nervous system as well as in peripheral neuronal circuits. In the peripheral nervous system substance P is found in the primary afferent neurons (A-delta pain fibres and C fibres), that are involved in the transmission of noxious stimuli. Bioactive substance P has been infused

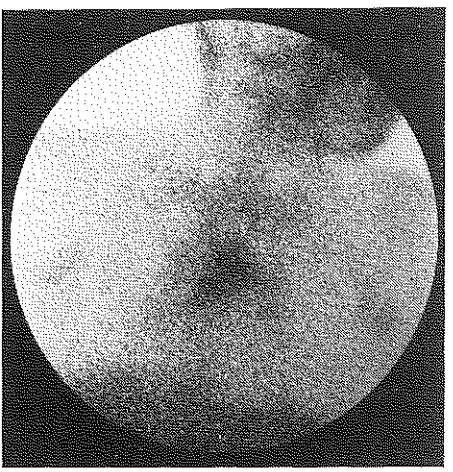

Figure 4. Accumulation of radioactivity in the thymus and salivary glands of a patient with liver sarcoidosis, 24 hours after injection of [^{111}In-DTPA-Arg^{1}]-substance P (anterior view).

intravenously in humans in doses of 0.5-1.5 pmol/kg per minute over 60 minutes or 0.5-8.0 pmol/kg per minute over 20 minutes without serious side effects (21,22). A very short half-life of 1.6 minute was measured, indicating a very high metabolic clearance. In normal plasma substance P is degraded by enzymatic hydrolysis involving multiple proteases (23).

In this study the [DTPA-Arg^{1}]-substance P was injected in patients with immune mediated diseases. In one out of twelve patients hypotension was observed with the clinical impression, that a vasovagal collapse caused the hypotension. After administration of [^{111}In-DTPA-Arg^{1}]-substance P a transient flush was observed in all patients.

A very short half-life of 10 minutes for plasma radioactivity and of 4 minutes for the intact radiopharmaceutical was measured. Radioactivity was excreted almost exclusively by the kidneys resulting in a low accumulation of radioactivity in the intestinal tract. Despite the high degradation rate of this radiopharmaceutical it could visualize affected tissues like the thymus and the salivary glands.

In eight patients with immune mediated diseases the thymus was visualized. In the control patients with cancer no thymus could be visualized, suggesting a high receptor expression in immune mediated diseases. Substance P receptors

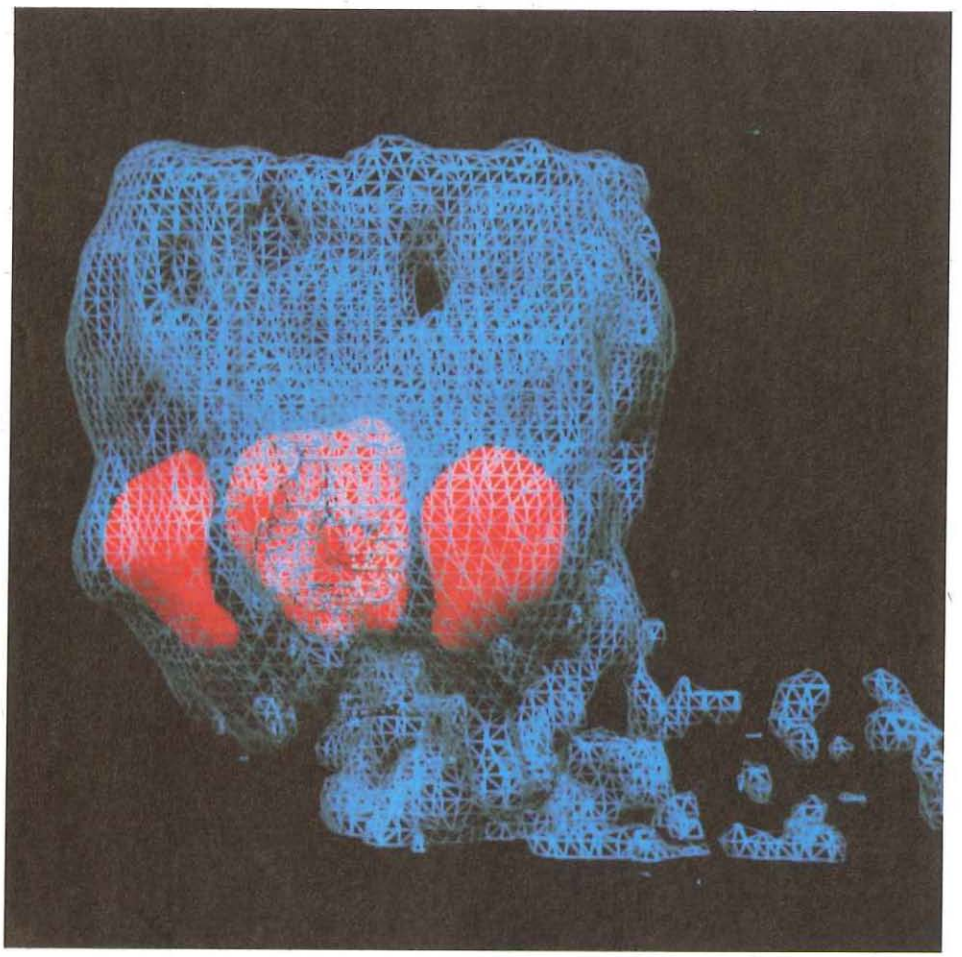

Figure 5. Three-dimensional representation of the parotid glands and the nose in a patient with Sjögren's syndrome, by ^{111}In labelled substance P analogue scintigraphy (24 hours after injection).

have been described recently to be present in the rat thymus, but their role is still obscure. Substance P has been identified in nerve fibres of sensory origin in the thymus (18). The outer cortex of the human thymus contains an 1-2 cell thick layer that is immunoreactive with antisera against substance P. In this region the most immature and recently migrated thymocytes are found emphasizing the role of neuropeptides in regulating the microenvironment for T cell development (16). The gene encoding both substance P and neurokinin A

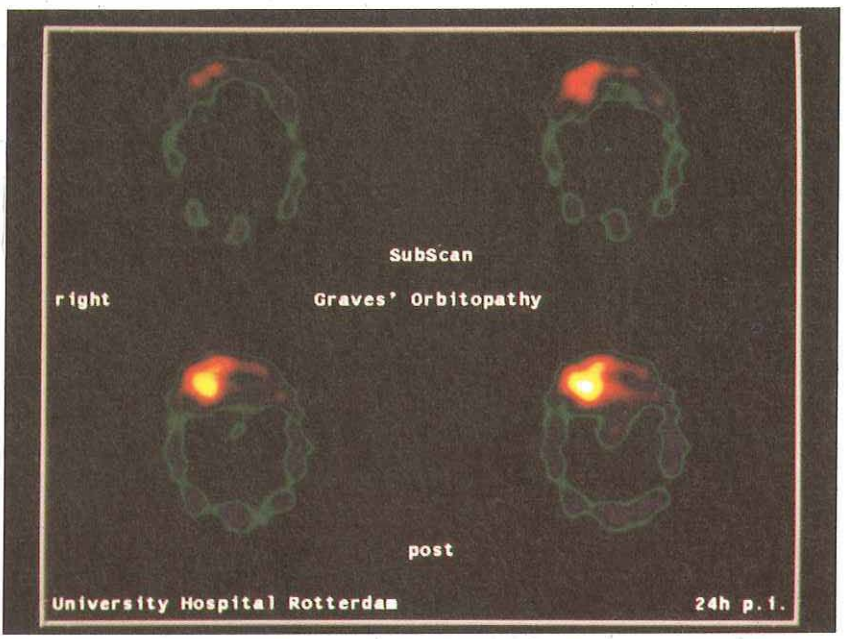

Figure 6. Uptake of radioactivity in a patient with unilateral ophthalmic Graves' disease, visualized by single photon emission computed tomography (SPECT) imaging, 24 hours after injection of [^{111}In-DTPA-Arg^{1}]-substance P.

was shown to be expressed in rat thymus, especially in a subpopulation of cells in the medulla (24).

The thymus is known to play an important role in myasthenia gravis. The negative scintigram in one of the patients with myasthenia gravis may be related to the use of high dose corticosteroids, which are known to cause involution of the thymus. Microscopy of thymuses of patients with myasthenia gravis revealed in 70% a follicular hyperplasia of the medulla, containing germinal centres resembling a stimulated lymph node (25,26). It may be that primary sensitisation against the acetylcholine receptor, which plays a crucial role in myasthenia gravis, occurs in the thymus. The reason why substance P receptors are present in the thymus of patients with immune mediated disease, as suggested in our patients, is obscure. However, it may mean that a similar process of sensitisation as is hypothesized in myasthenia gravis takes place in other autoimmune diseases as well.

Besides the thymus, inflammation sites in two patients with inflammatory bowel disease, the affected orbit in ophthalmic Graves' disease, the salivary glands in Sjögren's syndrome and the joints in rheumatoid arthritis were visualized. Although in patients with inflammatory bowel disease accumulation of radioactivity due to gut stenosis cannot be ruled out, the very low excretion of [^{111}In-DTPA-Arg^1]-substance P into the bowel (<0.05%) does not favour this explanation. Therefore, this accumulation is most likely due to the presence of substance P receptors at the sites of inflammation in these patients.

Substance P is supposed to be involved in inflammatory bowel disease. Upregulation of receptor binding sites at venules and muscle was previously shown by autoradiography (2). In these patients a high expression of substance P receptors was observed in comparison with control patients (1000-2000 times normal).

Substance P receptors were described in inflammatory synovitis in rats and humans (5,6). ^{125}I labelled substance P, binding to microvascular endothelium, paralleled the distribution of substance P immunoreactive nerves and had the characteristics of the neurokinin 1 receptor (6).

In this study we present our preliminary results concerning the use of [^{111}In-DTPA-Arg^1]-substance P as a new radiopharmaceutical with a very short half-life in the circulation. The thymus, which is usually involuted after puberty, expressed enough substance P receptors to allow in vivo visualization. The role of the thymus in autoimmune diseases is obscure; only enlargement of the thymus has been described in these diseases. Further investigations are necessary to evaluate the role of substance P receptor expression in the thymus in these diseases. Also, inflammatory sites in various diseases could be visualized by substance P receptor scintigraphy.

References

1. Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J Biol Chem 1970;245:4784-90.

2. Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EP Jr, Vigna SR, Maggio JE, Kruger L, Mantyh PW. Receptor binding sites for substance P, but not substance K, or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens from patients with ulcerative colitis and Crohn disease. Proc Natl Acad Sci U S A 1988;85:3235-9.
3. Mantyh PW. Substance P and the inflammatory and immune response. Ann N Y Acad Sci 1991;632:263-71.
4. Walsh DA, Mapp PI, Wharton J, Rutherford RAD, Kidd BL, Revell PA, Blake DR, Polak JM. Localisation and characterisation of substance P binding to human synovial tissue in rheumatoid arthritis. Ann Rheum Dis 1992;51:313-7.
5. Joyce TJ, Yood RA, Carraway RE. Quantitation of substance-P and its metabolites in plasma and synovial fluid from patients with arthritis. J Clin Endocrinol Metab 1993;77:632-7.
6. Walsh DA, Salmon M, Mapp PI, Wharton J, Garrett N, Blake DR, Polak JM. Microvascular substance P binding to normal and inflamed rat and human synovium. J Pharmacol Exp Ther 1993:267:951-60.
7. Walsh DA, Salmon M, Wharton J, Mapp PI, Polak JM. Autoradiographic localisation and characterisation of substance P binding sites in rat knees. Regul Pept 1993;46:189-92.
8. Foreman JC, Jordan CC. Histamine release and vascular changes induced by neuropeptides. Agents Actions 1983;13:105-16.
9. Payan DG, Brewster DR, Missirian-Bastian A, Goetzl EJ. Substance P recognition by a subset of human T lymphocytes. J Clin Invest 1984;74:1532-9.
10. Ruff MR, Wahl SM, Pert CB. Substance P receptor mediated chemotaxis of human monocytes. Peptides 1985;6 Suppl 2:107-11.
11. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
12. Rosai J, Levine GD. Tumors of the thymus. In: Atlas of tumor pathology, sec ser, fasc 13. Armed Forces Institute of Pathology, Washington DC. 1976;26-33:133-7.
13. Levine GD, Rosai J. Thymic hyperplasia and neoplasia: a review of current concepts. Hum Pathol 1978;9:495-515.
14. Otto HF. Pathology des Thymus. Springer, Berlin Heidelberg New York, 106-115:186-8.
15. Tridente G. Immunopathology of the human thymus. [Review] Semin Hematol 1985;22:56-67.
16. Piantelli M, Maggiano N, Larocca LM, Ricci R, Ranelletti FO, Lauriola L, Capelli A. Neuropeptide-immunoreactive cells in human thymus. Brain Behav Immun 1990;4:189-97.
17. Lorton D, Bellinger DL, Felten SY, Felten DL. Substance P innervation of the rat thymus. Peptides 1990;11:1269-75.
18. Shigematsu K, Saavedra JM, Kurihara M. Specific substance P binding sites in rat thymus and spleen: in vitro autoradiographic study. Regul Pept 1986;16:147-56.
19. Bakker WH, Albert R, Bruns C, Breeman WAP, Hofland LJ, Marbach P, Pless J, Pralet D, Stolz B, Koper JW, Lamberts SWJ, Visser TJ, Krenning EP. [^{111}In-DTPA-D-Phe^{1}]-octreotide, a potential radiopharmaceutical for imaging of somatostatin receptor-positive tumors: synthesis, radiolabeling and in vitro validation. Life Sci 1991;49:1583-91.

20. Breeman WAP, Hofland LJ, van der Pluijm M, van Koetsveld PM, de Jong M, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Visser TJ, Lamberts SWJ, Krenning EP. 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. Eur J Nucl Med 1994;21:328-35.
21. Coiro V, Capretti L, Volpi R, Davoli C, Marcato A, Cavazzini U, Caffarri G, Rossi G, Chiodera P. Stimulation of ACTH/cortisol by intraveneously infused substance P in normal men: Inhibition by sodium valproate. Neuroendocrinology 1992; 56:459-63.
22. Schaffalitzky De Muckadel OB, Aggestrup S, Stentoft P. Flushing and plasma substance P concentration during infusion of synthetic substance P in normal man. Scand J Gastroenterol 1986;21:498-502.
23. Mussap CJ, Gerargthy DP, Burcher E. Tachykinin receptors: a radioligand binding perspective. [Review] J Neurochem 1993;60:1987-2009.
24. Ericsson A, Geenen V, Robert F, Legros JJ. Expression of preprotachykinin-A and neuropeptide-Y messenger RNA in the thymus. Molecular Endocrinology 1990;4:1211-8.
25. Vincent A, Newson-Davis J, Newton P, Beck N. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. Neurology 1983;33:1276-82.
26. Kuks JBM, Oosterhuis HJGH, Limburg PC, The TH. Anti-acetylcholine receptor antibodies decrease after thymectomy in patients with myasthenia gravis. Clinical correlations. J Autoimmun 1991;4:197-211.

CHAPTER 9

SOMATOSTATIN AND SUBSTANCE P ANALOGUES:

APPLICATIONS IN AUTOIMMUNE AND HAEMATOLOGICAL DISEASES

Neuroimmunoendocrinology

Neuroimmunoendocrinology is a rapidly evolving field, which until recently has had only limited clinical applications. A well-known link between the immune system and the neuroendocrine system at the molecular level is formed by the glucocorticoids, which play an important role in the suppression of a hyperactive reacting immune system, for example in systemic lupus erythematosus. On the other hand, however, adrenalectomized animals are functionally immunosuppressed (1). Besides corticosteroids, many neuroendocrine peptides are involved in both the regulation of the immune system and the bidirectional communication between the immune and neuroendocrine systems.

Somatostatin

The modulation of immune functions by somatostatin

Somatostatin receptors are known to be expressed by human lymphoid cell lines, normal activated lymphocytes, and malignant lymphoid cells (2). They have been identified in the human thymus and spleen, and within the germinal centres of gastrointestinal associated lymphoid tissue (3,4). These observations led to the suggestion that somatostatin receptor expression might be related to an activated and/or proliferative state of lymphoid cells. Somatostatin might modulate the immune response by a variety of mechanisms. These include modification of immunoglobulin secretion by plasma cells, suppression or enhancement of lymphocyte proliferation, cytotoxicity and changes in cytokine production, release of mediators by basophils, recruitment of eosinophils, and changes in macrophage function.

Somatostatin inhibits all three classes of immunoglobulin production by unstimulated as well as pokeweed mitogen stimulated lymphocytes (5,6). The highest percentage of inhibition was observed in the IgG and IgA synthesis.

Somatostatin analogues have direct antiproliferative effects on a variety of experimental human solid tumour cell lines (7). The influence of somatostatin on lymphocyte and lymphoid cell line proliferation was extensively studied using cells originating from different species. Result of these studies were summarized in a recently published review (2,this thesis). Most investigations reviewed (10

out of 16) showed a concentration dependent modulation of the spontaneous and lectin induced proliferation response of lymphocytes and cell lines by somatostatin (2). In particular, an inhibitory effect on the proliferation was observed in the range of 10 pM - 1 nM. In three out of 16 reports, a biphasic proliferation response of lymphoid cells was observed. This response was antimitotic at low somatostatin concentrations, but became stimulating at concentrations above 0.1 μM. At present, the pathophysiological meaning of this biphasic response is not known.

In vivo administration of somatostatin and the somatostatin analogue BIM-23014c affects the natural killer activity in vitro of lymphocytes from mouse spleen and Peyer's patches (8). At nanomolar concentrations somatostatin inhibits the release of lymphotoxin and tumour necrosis factor α (9). Somatostatin was shown to inhibit interferon γ by granuloma and splenic T lymphocytes by as much as 75% (10,11). Somatostatin may be involved in hyperreactivity by regulation of mediator release by basophils and the recruitment of eosinophils; at low somatostatin concentrations an inhibitory effect was observed (12-13).

Somatostatin receptors in malignant lymphomas

In non-Hodgkin's lymphoma of low, intermediate and high grade malignancy somatostatin receptors have been detected with in vitro autoradiography in, respectively, 92%, 100% and 70% of the patients (this thesis). Non-Hodgkin's lymphoma and Hodgkin's disease could be visualized with in vivo somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^{1}]-octreotide. This radioligand is strongly related to octreotide, and binds with high affinity to two of the five known somatostatin receptor subtypes, i.e. receptor subtypes 2 and 5, and with low affinity to receptor subtype 3 (14). Somatostatin receptor scintigraphy was found to be a sensitive method for the staging of malignant lymphomas, and revealed an upgrade in the clinical stage in six of 40 patients with Hodgkin's disease, when compared to the use of conventional staging methods alone. In 17 out of 62 patients with non-Hodgkin's lymphoma somatostatin receptor scintigraphy disclosed lymphoma localizations that were not detected by conventional staging methods, resulting in an upgrade of the clinical stage in 13 patients. However, scintigraphy failed to demonstrate selected sites in five

patients with Hodgkin's disease and in 14 patients with non-Hodgkin's lymphoma.

Somatostatin receptors in granulomatous diseases

Somatostatin receptors are expressed in human granulomatous diseases. These receptors have been detected by autoradiography in tissue biopsies from patients with sarcoidosis and human tuberculosis, and were predominantly located in the epithelioid cell regions (this thesis). Biopsy of a lymph node of a patient with sarcoidosis who had been treated successfully with corticosteroids showed no somatostatin receptor expression. Histopathological examination of this lymph node showed complete sclerosis.

The role of neuropeptides in granulomatous inflammation was extensively studied by Weinstock et al. (15-21). Eosinophils and macrophages in granulomas induced by *Schistosoma mansoni* eggs secrete neuropeptides. These granuloma macrophages produce somatostatin 14, but not somatostatin 28. Somatostatin receptors in granulomas from murine schistosomiasis were found to be displayed exclusively by a subset of granuloma CD4 positive T lymphocytes. These data are in contrast with our observations in granulomas from patients with sarcoidosis and tuberculosis. Somatostatin receptors in these human granulomas were predominantly located in the epithelioid cell regions.

In almost all patients with granulomatous diseases, like sarcoidosis, tuberculosis, aspergillosis and Wegener's granulomatosis, inflammation localizations could be visualized with somatostatin receptor scintigraphy (this thesis). Positive scintigraphy in sarcoidosis seems to be related to the efficacy of therapy. These findings suggest a relation between the activity of granulomatous disease and the expression of somatostatin receptors.

Somatostatin receptors in rheumatoid arthritis and Sjögren's syndrome

Somatostatin receptors were found to be localized in blood vessels of diseased synovial tissues of patients suffering from rheumatoid arthritis (this thesis). The receptors were homogeneously distributed in the whole wall of veins but could not be identified in arteries. The fact that the venous wall rarely contained

non-vascular constituents such as infiltrating leucocytes, indicates that the muscular wall and perhaps the endothelium are the most likely sites of somatostatin receptor expression in rheumatoid arthritis. Displacement experiments with somatostatin 14, somatostatin 28, and octreotide suggest that the receptors expressed are subtype 2 receptors. Affected joints in patients with rheumatoid arthritis could be visualized with in vivo somatostatin receptor scintigraphy, using ^{111}In labelled octreotide as radioligand. The clinical degree of inflammation correlated closely with the degree of uptake of [^{111}In-DTPA-D-Phe^{1}]-octreotide. Uptake of radioactivity was also found in the affected joints of patients with systemic lupus erythematosus, sarcoidosis and Crohn's disease. In nine out of 12 patients with Sjögren's syndrome the affected salivary glands, associated lymph nodes or affected lungs were visualized. In Sjögren's syndrome the salivary glands are infiltrated with foci of CD4 positive lymphocytes.

All the diseases described above are characterized by the involvement of malignant lymphoid cells, activated lymphocytes and monocytes, or by vasculitis. Normal lymph nodes as well as acute bacterial infections cannot be visualized by octreotide scintigraphy, while the normal spleen is visualized in all patients. Activation or malignant transformation of immunocompetent cells results in the expression of high affinity somatostatin receptors, which can be visualized as localizations of tumour or inflammation.

Substance P

Modulation of the immune system by substance P

Substance P is another neuropeptide that is extensively involved in immune related diseases. Substance P modulates several immune and hypersensitivity activities. It has been reported to modulate the functional activities of inflammatory cells by causing degranulation of mast cells, stimulation of lysosomal enzyme release from neutrophils, enhancement of neutrophil chemotaxis and phagocytosis, and activation of macrophages (22-26). Substance P has also been shown to stimulate T lymphocyte proliferation, to enhance the effect of T cell mitogens and the production of immunoglobulins by plasma cells (27-30). Substance P binds to specific receptors on rat tissue mast cells to induce histamine and leukotriene release at micromolar concentrations

(22). The hypersensitivity effects, such as vasodilatation and alteration of the microvascular permeability appear to be mediated by substance P induced factors such as histamine and leukotrienes. There are several reports showing that substance P is involved in cytokine production. Substance P stimulates the induction of interleukin 2 (31,32), interleukin 1, interleukin 6, and tumour necrosis factor α (33). Substance P is present in nerve fibres that innervate the spleen, thymus and lymph nodes, and in nerve fibres that are in close contact with the mast cells in the intestine. This supports the concept that substance P influences the activity of the immune system (34-36).

Substance P in inflammatory conditions

Several lines of evidence indicate that tachykinin neuropeptides like substance P play a role in the inflammatory and immune response, both in peripheral tissues, such as the lungs, joints and colon, as well as in the central nervous system. Glial cells in vivo express high concentrations of substance P receptors after transection of retinal ganglion cell axons (37). Receptor binding sites for substance P are expressed at high concentrations in arterioles, venules and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease (38). It has also been shown, that there is a large increase in substance P receptors in rheumatoid synovium compared with normal synovium (39,40). It is now widely accepted that there is a neurogenic mechanism in the pathophysiology of arthritis, and substance P may be one of the important mediators.

Structural changes in the substance P molecule, namely the coupling of a DTPA group, allowed the convenient production of a radiopharmaceutical, despite its short half-life. With this compound we were able to visualize the involved joints in rat adjuvant arthritis (this thesis). In man uptake of radioactivity was found in the thymus of patients with immune mediated diseases like Sjögren's syndrome. The precise role of substance P receptor expression in the thymus is not clear. However, the expression of a neuropeptide receptor in an organ that is involved in the immune regulation suggests an interaction between the neuroendocrine and the immune system. Apart from the thymus, we found uptake of substance P bound radioactivity in the parotid glands in Sjögren's syndrome, and in the affected eyes in ophthalmic Graves' disease (this thesis).

Somatostatin analogues and substance P antagonists in the treatment of autoimmune diseases?

In vitro somatostatin and somatostatin analogues have inhibitory effects on the immune system, such as inhibition of lymphocyte proliferation, immunoglobulin synthesis, cytokine secretion, histamine release and monocyte chemotaxis as mentioned above (2). Results of studies on somatostatin analogue therapy in 'autoimmune disease' in animal models are still preliminary. Important results are reported by Weinstock et al., who found a reduction of the granulomatous response in *Schistosoma mansoni* egg induced murine hepatic granulomas by octreotide treatment, presumably because of decrease of the interferon γ production (10). Further, the somatostatin agonists BIM-23014 and octreotide suppress the in vivo inflammatory reaction in aseptic subcutaneous carrageenin induced inflammation in rat (41). A reduction of the volume and the leucocyte concentration of the exudate was reported. Immunohistochemical evaluation of the local mediators substance P, tumour necrosis factor α and corticotropin releasing factor showed a significant inhibitory effect of these mediators.

Only preliminary data are available on the use of somatostatin analogues in humans. A beneficial effect of somatostatin injected intraarticularly in patients with rheumatoid arthritis has been reported (42-44). We found a beneficial effect during short treatment with systemic octreotide in a patient with arthritis (unpublished data). In psoriasis the use of octreotide has resulted in a decrease in the skin thickness (45). Furthermore, octreotide was successfully used in the treatment of diarrhoea in patients with systemic sclerosis (46). Octreotide showed to have a beneficial response in the treatment of patients with low grade non-Hodgkin's lymphoma (47). Recently, a patient with intestinal lymphangiectasia, who had been steroid dependent for years, was successfully treated with octreotide monotherapy (48). The latter observation may be related to the expression of somatostatin receptors on veins and venules in inflammatory tissue (49). Modulation of the vascular somatostatin receptors may result in a decrease in the inflammation associated extravasation of plasma and leucocytes. Moreover, octreotide has been found to decrease the mucosal damage in experimental colitis, and somatostatin receptors are known to be expressed on the mucosal veins and venules in inflammatory bowel disease (49,50).

Substance P has antagonistic effects on the immune system, when compared with somatostatin. Substance P is supposed to be an activator of the immune system. Inhibition of substance P by specific antibodies was found to decrease the inflammatory activity in experimental murine intestinal inflammation (51). Besides, the non-peptide substance P antagonist CP96,345 inhibits the inflammation completely in this model with an increasing substance P tissue level (52). Octreotide treatment of this intestinal inflammation resulted also in a significant decrease of the inflammatory activity and a decreased substance P tissue level (52). Peripheral localized inflammation induces increased substance P synthesis and transport towards to the inflammatory tissue (53). Somatostatin is known to inhibit the release of substance P. This may result in a decreased inflammatory activity, apart from the primary effect of somatostatin on the immune system (50,54,55). Furthermore, like octreotide, CP96,345 decreases the granuloma size after intravenous administration, possibly by inhibiting the secretion of interferon γ (56). The secretion of interferon γ was unaffected when both substance P and somatostatin were added to the medium, suggesting opposite effects of the two peptides. Substance P receptors are expressed in both arteries and veins in inflammatory tissue (38-40). These vascular receptors may be a target for treatment as well. Substance P produces a dose dependent, transient vasodilatation of the rat knee joint microvasculature, which can be reduced by CP96,345 (57).

Human lymphoid cells were found to selectively express somatostatin receptor subtype 2, which makes these cells a target for somatostatin analogues with high affinity to somatostatin receptor subtype 2, like octreotide, RC-160 and BIM-23014 (this thesis).

In conclusion, the somatostatin analogue octreotide and, in the future, substance P antagonists may be of use in the treatment of inflammatory diseases, like sarcoidosis, rheumatoid arthritis and inflammatory bowel disease. The neuropeptide receptor status in these patients, which can be obtained by peptide receptor scintigraphy may be related to and predictive for the efficacy of therapy.

References

1. Blalock JE, Bost KL. Neurimmunoendocrinology. In: Progress in allergy. Kerger - Basel - New York, 1988.
2. van Hagen PM, Krenning EP, Kwekkeboom DJ, Reubi JC, van den Anker-Lugtenburg PJ, Löwenberg B, Lamberts SWJ. Somatostatin and the immune and haematopoietic system; a review. Eur J Clin Invest 1994;24:91-9.
3. Reubi JC, Horisberger U, Waser B, Gebbers JO, Laissue JA. Preferential location of somatostatin receptors in germinal centers of human gut lymphoid tissue. Gastroenterology 1992;103:1207-14.
4. Reubi JC, Waser B, Horisberger U, Krenning EP, Lamberts SWJ, Gebbers JO, Gersbach P, Laissue JA. In vitro autoradiographic and in vivo scintigraphic localization of somatostatin receptors in human lymphatic tissue. Blood 1993;82:2143-51.
5. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. Distribution of somatostatin receptors on murine spleen and Peyer's patch T and B lymphocytes. Brain Behav Immun 1987;1:173-84.
6. Scicchitano R, Dazin P, Bienenstock J, Payan DG, Stanisz AM. The murine IgA-secreting plasmacytoma MOPC-315 expresses somatostatin receptors. J Immunol 1988;141:937-941.
7. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. [Review] Endocr Rev 1991;12:450-82.
8. Agro A, Padol I, Stanisz AM. Immunomodulatory activities of the somatostatin analogue BIM 23014c: effects on murine lymphocyte proliferation and natural killer activity. Regul Pept 1991;32:129-39.
9. Yousefi S, Ghazinouri A, Vaziri N, Tilles J, Carandang G, Cesario T. The effect of somatostatin on the production of human interferons by mononuclear cells. Proc Soc Exp Biol Med 1990;194:114-8.
10. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. Clin Immunol Immunopathol 1992;64:17-22.
11. Blum AM, Metwali A, Mathew RC, Cook G, Elliott D, Weinstock JV. Granuloma T lymphocytes in murine schistosomiasis mansoni have somatostatin receptors and respond to somatostatin with decreased IFN-gamma secretion. J Immunol 1992;149:3621-6.
12. Goetzl EJ, Payan DG. Inhibition by somatostatin of the release of mediators from human basophils and rat leukemic basophils. J Immunol 1984;133:3255-9.
13. Etienne A, Soulard C, Thonier F, Braquet P. Modulation of eosinophil recruitment in the rat by the platelet activating factor (PAF) antagonist, BN 52021, the somatostatin analog BIM 23014, and by cyclosporin A. Prostaglandins 1989;37:345-57.
14. Weckbecker G, Raulf F, Stolz B, Bruns C. Somatostatin analogs for diagnosis and treatment of cancer. [Review] Pharmacol Ther 1993;60:245-64.
15. Weinstock JV. Production of neuropeptides by inflammatory cells within the granulomas of murine schistosomiasis mansoni. [Review] Eur J Clin Invest 1991; 21:145-53.
16. Weinstock JV, Blum AM, Malloy T. Macrophages within the granulomas of murine schistosoma mansoni are a source of a somatostatin 1-14-like molecule. Cell Immunol 1990;131:381-90.
17. Weinstock JV, Blum AM. Tachykinin production by granulomas of murine schistosomiasis mansoni. J Immunol 1989;142:3256-61.

18. Weinstock JV, Blum AM, Walder J, Walder R. Eosinophils from granulomas in murine schistosomiasis mansoni produce substance P. J Immunol 1988;141:961-6.
20. Weinstock JV, Blum AM. Detection of vasoactive intestinal peptide and localization of its mRNA within granulomas of murine schistosomiasis. Cell Immunol 1990;125:291-300.
21. Weinstock JV. Neuropeptides and the regulation of granulomatous inflammation. [Review] Clin Immunol Immunopathol 1992;64:17-22.
22. Goetzl EJ, Chernov T, Renold F, Payan DG. Neuropeptide regulation of the expression of immediate hypersensitivity. J Immunol 1985;135 Suppl:S802-5.
23. Bar-Shavit Z, Goldman R, Stabinsky Y, Gottlieb P, Fridkin M, Teichberg VI, Blumberg S. Enhancement of phagocytosis - a newly found activity of substance P residing in its N-terminal tetrapeptide sequence. Biochem Biophys Res Commun 1980;94:1445-51.
24. Marasco WA, Showell HJ, Becker EL. Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. Biochem Biophys Res Commun 1981;99:1065-72.
25. Mazurek N, Pecht I, Teichberg VI, Blumberg S. The role of the N-terminal tetrapeptide in the histamine releasing action of substance P. Neuropharmacology 1981;20:1025-7.
26. Hartung HP, Wolters K, Toyka KV. Substance P: binding properties and studies on cellular responses in guinea pig macrophages. J Immunol 1986;136:3856-63
27. Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. J Immunol 1983:131;1613-5.
28. Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. J Immunol 1986;136:152-6.
29. Reusch MK, Karasek MA, Nickoloff BJ. Effect of neuropeptides present in skin on the proliferation of human peripheral mononuclear cells and T cells. Arch Dermatol Res 1988;280:279-81.
30. Roberts AI, Taunk J, Ebert EC. Human lymphocytes lack substance P receptors. Cell Immunol 1992;141:457-65.
31. Rameshar P, Gascon P, Ganea D. Immunoregulatory effects of neuropeptides. Stimulation of interleukin-2 production by substance P. J Neuroimmunol 1992;37:65-74.
32. Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T cells. J Immunol 1992;148:3498-504.
33. Lotz M, Vaughan JH, Carson DA. Effects of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988;241:1218-21.
34. Weihe E, Muller S, Fink T, Zentel HJ. Tachykinins, calcitonin gene-related peptide, and neuropeptide Y in nerves in the mammalian thymus: interaction with mast cells in autonomic and sensory neuroimmunomodulation? Neurosci Lett 1989;100:77-82.
35. Bellinger DL, Lorton D, Romano TD, Olschowka JA, Felten SY, Felten DL. Neuropeptide innervation of lymphoid organs. [Review] Ann N Y Acad Sci 1990;594:17-33.
36. Bienenstock J, Tomioka M, Matsuda H, Stead RH, Quinonez G, Simon GT, Coughlin MD, Denburg JA. The role of mast cells in inflammatory processes: evidence of nerve/mast cell interactions. Int Arch Allergy Appl Immunol 1987;82:238-43.
37. Mantyh PW, Johnson DJ, Boehmer CG, Catton MD, Vinters HV, Maggio JE, Too HP, Vigna SR. Proc Natl Acad Sci U S A 1989;86:5193-7.

38. Mantyh CR, Gates TS, Zimmerman RP, Welton ML, Passaro EP Jr, Vigna SR, Maggio JE, Kruger L, Mantyh PW. Receptor binding sites for substance P, but not substance K or neuromedin K, are expressed in high concentrations by arterioles, venules, and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn disease. Proc Natl Acad Sci U S A 1988;85;3235-9.
39. Mapp PI, Walsh DA, Cruwys SC, Kidd BL, Blake DR. Receptors to the neuropeptide substance P in human synovium. Br J Rheumatol 1990;29 Suppl 1:44.
40. Walsh DA, Mapp PI, Wharton J, Rutherford RAD, Kidd BL, Revell PA, Blake DR, Polak JM. Localisation and characterisation of substance P binding to human synovial tissue in rheumatoid arthritis. Ann Rheum Diss 1992;51:313-7.
41. Karalis K, Mastorakos G, Chrousos GP, Tolis G. Somatostatin analogues suppress the inflammatory reaction in vivo. J Clin Invest 1994;93:2000-6.
42. Agro A. Stanisz AM. Are lymphocytes a target for substance P modulation in arthritis? Semin Arthritis Rheum 1992;21:252-8.
43. Matucci-Cerinic M, Marabini S. Somatostatin treatment for pain in rheumatoid arthritis: a double blind versus placebo study in knee involvement. Med Sci Res 1988;16:233-4.
44. Ciocci A, Coari G, Di Franco M, Di Novi MR, Mauceri MT. Intra-articular treatment with somatostatin 14 in rheumatoid arthritis. Clin Ter 1994;145:463-7.
45. Camissa C, Bagatell F, Bainbridge C. Somatostatin analogue (octreotide acetate) vs. placebo in the treatment of psoriasis: a multicenter study. J Invest Derm 1990;94:511.
46. Witzig TE, Letendre L, Gerstner J, Schroeder G, Maillaird JA, Colon-Otero G, Marschke RF, Windschitl HE. Evaluation of a somatostatin analog in the treatment of lymphoproliferative disorders: results of a phase II North Central Cancer Treatment Group trial. J Clin Oncol 1995;13:2012-5.
47. Soudah HC, Hasler WL, Owyang C. Effect of octreotide on intestinal motility and bacterial overgrowth in scleroderma. N Engl J Med 1991;325:1461-7.
48. Bac DJ, van Hagen PM, Postema PTE, ten Bokum AM, Zondervan PE, van Blankenstein M. Octreotide for protein-losing enteropathy with intestinal lymphangiectasia [letter]. Lancet 1995;345:1639
49. Reubi JC, Mazzucchelli L, Laissue JA. Intestinal vessels express a high density of somatostatin receptors in human inflammatory bowel disease. Gastroenterology 1994;106:951-9.
50. Eliakim R, Karmeli F, Okon E, Rachmilewitz D. Octreotide effectively decreases mucosal damage in experimental colitis. Gut 1993;34:264-9.
51. Agro A, Stanisz AM. Inhibition of murine intestinal inflammation by anti-substance P antibody. Reg Immunol 1993;5:120-6
52. Stanisz AM. Neuroimmunomodulation in the gastrointestinal tract. [Review] Ann N Y Acad Sci 1994;741:64-72
53. Donnerer J, Schuligoi R, Stein C, Amann R. Upregulation, release and axonal transport of substance P and calcitonin gene-related peptide in adjuvant inflammation and regulatory function of nerve growth factor. [Review] Regul Pept 1993:46;150-4.
54. Pernow B. Role of tachykinins in neurogenic inflammation. J Immunol 1985;135:S812-5.
55. Furchgott RF. Role of endothelium in the responses of vascular smooth muscle. [Review] Circ Res 1983;53:557-73.

56. Blum AM, Metwali A, Cook G, Mathew RC, Elliott D, Weinstock JV. Substance P modulates antigen-induced, IFN-gamma production in murine schistosomiasis mansoni. J Immunol 1993;151:225-33.
57. Lam FY, Ferrell WR. Effects of interactions of naturally-occurring neuropeptides on blood flow in the rat knee joint. Br J Pharmacol 1993;108:694-9.

SUMMARY

This thesis describes the results of several studies on somatostatin and substance P receptor expression in autoimmune diseases and haematological malignancies. Receptor studies were performed by in vitro receptor autoradiography, ligand binding assays, and reverse transcriptase polymerase chain reaction techniques, and by in vivo receptor scintigraphy with the ^{111}In labelled somatostatin analogue octreotide or ^{111}In labelled substance P.

The biological aspects of somatostatin are summarized in Chapter 1. Somatostatin is a 14 or 28 amino acid peptide which is found throughout the human body, but mainly in the endocrine glands and central nervous system. Recently, five subtypes of somatostatin receptors have been identified, which are all coupled to a G protein. Somatostatin acts as a neurohormone and neurotransmitter with a generally inhibitory effect. Long-acting somatostatin analogues, like octreotide, are clinically used in the treatment of hormone production by endocrine tumours. Radiolabelled octreotide is successfully used in the visualization of tumours derived from the amine precursor uptake and decarboxylation (APUD) system. Physiological uptake of this radioimager is found in the thyroid, liver, pituitary, spleen, kidneys and urinary bladder. Deposition of radioactivity at other sites is supposed to be pathological.

In Chapter 2, the relation between somatostatin and the immune system is reviewed. Somatostatin and somatostatin receptors have been demonstrated in non-pathological and pathological lymphoid tissue and may play a regulatory, mostly inhibitory, role in the immune response. Somatostatin is produced by lymphocytes and monocytes, suggesting an autocrine or paracrine regulatory role, but it may also be released by nerve endings. It can therefore be hypothesized that, apart from a role of somatostatin in local immunomodulation of cells belonging to the immune system, a second pathway exists in which this peptide exerts its effects via neuroendocrine modulation of the immune response. This might represent a direct regulatory relation between the nervous and the immune system.

In Chapter 3 the somatostatin receptor status is evaluated in malignant lymphomas that had been surgically removed from 31 patients. Somatostatin receptors were visualized by in vitro somatostatin receptor autoradiography with the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide as radioligand. Of eleven patients with low grade malignancy B cell non-Hodgkin's lymphoma ten patients were somatostatin receptor positive, with a high receptor density restricted to

the neoplastic follicles. All of the eight intermediate grade non-Hodgkin's lymphomas were somatostatin receptor positive. Seven out of ten non Hodgkin's lymphomas of high grade malignancy were somatostatin receptor positive, often with a high density of receptors. Somatostatin receptors were of high affinity and were specific for bioactive somatostatin analogues. Somatostatin receptor scintigraphy, using [^{111}In-DTPA-D-Phe^{1}]-octreotide as radioligand, was used in ten consecutive patients with malignant lymphomas (Hodgkin's disease or non-Hodgkin's lymphoma). In all patients the lymphoma deposits could be visualized. In four patients, additional tumour localizations were observed as compared to the results of combined physical and radiological examinations (computer tomography and ultrasonography). In three cases tissue biopsies were taken and confirmed by autoradiography to be somatostatin receptor positive. These data indicate that malignant lymphomas may express somatostatin receptors in sufficient numbers and density to allow in vivo tumour visualization with a radiolabelled somatostatin analogue. Recently, the overall sensitivity in larger series revealed a sensitivity rate for Hodgkin's disease and non-Hodgkin's lymphoma of 97.5% and 85%, respectively. This resulted in an upgrade of clinical stage in 15% of the patients with Hodgkin's disease and in 21% of the patients with non-Hodgkin's lymphoma.

In Chapter 4 the results on somatostatin receptor expression in granulomatous diseases are presented. Twenty consecutive patients were investigated, 12 patients with sarcoidosis, one with both sarcoidosis and aspergillosis, four with tuberculosis, and three with Wegener's granulomatosis. For in vivo somatostatin receptor imaging, total body scintigraphy was performed 24 and 48 hours after the administration of [^{111}In-DTPA-D-Phe ^{1}]-octreotide. Granuloma localizations were visualized in almost all patients studied. In vitro autoradiography of fresh tissue biopsies, using the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide, showed binding at sites that were microscopically identified as granulomatous inflammation. These observations demonstrate the expression of somatostatin receptors by human granulomas. This scintigraphy procedure may contribute to a more precise staging and evaluation of granulomatous diseases.

Chapter 5 describes the in vivo and in vitro investigations of somatostatin receptor expression on synovial membranes in patients with rheumatoid arthritis. The joints of 14 consecutive patients with active rheumatoid arthritis, four patients with severe osteoarthritis, and 30 control patients were studied. The somatostatin analogue [^{111}In-DTPA-D-Phe^{1}]-octreotide was used for in vivo

somatostatin receptor scintigraphy and the somatostatin analogue [^{125}I-Tyr^{3}]-octreotide was used for in vitro somatostatin receptor autoradiography. Seventy-six percent of both the painful and the swollen joints of the patients with rheumatoid arthritis were visualized. The degree of pain and swelling correlated well with positive scintigraphic findings in the joints. In patients with osteoarthritis the uptake of radioactivity in the affected joints was significantly lower than that in patients with rheumatoid arthritis. None of the joints of the control patients demonstrated uptake of radioactivity. The expression of somatostatin receptors in synovium from patients with rheumatoid arthritis was studied by in vitro receptor autoradiography. Receptors were absent in one case, a successfully treated patient with quiescent disease. Somatostatin receptors were of high affinity and specific for biologically active somatostatin analogues. Displacement by nanomolar concentrations of somatostatin 14, somatostatin 28, and octreotide was observed, suggesting that most of the receptors identified belong to the somatostatin receptor subtype 2. The somatostatin receptors were preferentially located in blood vessels, with specific labelling of the veins but not of the arteries. The whole vessel wall was homogeneously labelled, including the smooth muscle cells and probably the endothelium. These data suggest that the synovium in active rheumatoid arthritis expresses a high density of somatostatin receptors. Somatostatin may act through these venous receptors to influence the inflammatory process by induction of vasoconstriction, inhibition of plasma extravasation and cell migration, or inhibition of neovascularization.

In twelve patients with Sjögren's syndrome somatostatin receptor expression was found in the affected tissue. In nine patients uptake of radioactivity was found in the affected salivary glands, lungs, joints, and even in the heart. Somatostatin receptor scintigraphy enables the visualization of the extent of disease, and may be used in the monitoring of the efficacy of therapy in Sjögren's syndrome, especially in the case of lung involvement.

In Chapter 6 we described the expression of somatostatin receptor subtype messenger ribonucleic acid (mRNA) and membrane somatostatin receptor expression in human lymphoid cell lines. Recently, five somatostatin receptor subtypes have been identified. In this study the expression of mRNA for somatostatin receptor subtypes was investigated in ten B cell lines and eight T cell lines by reverse transcriptase polymerase chain reaction. Two B cell lines (JY and TMM) and two T cell lines (HSB-2 and MT-1) expressed mRNA for

somatostatin receptor subtype 2, resulting in the expression of membrane somatostatin receptors in the cell lines JY, TMM, and MT-1. These data indicate that only somatostatin receptor subtype 2 mRNA is expressed in lymphoid cells.

In Chapter 7 the role of substance P in immune mediated diseases is summarized. Substance P is a peptide of eleven amino acids, and is a member of the family of tachykinins. The neurokinin 1 receptor, which is present in both the central and peripheral nervous system, preferentially interacts with substance P. This neuropeptide modulates several immune and hypersensitivity activities. The substance P concentrations in serum and synovial fluid in patients with rheumatoid arthritis are increased. Also, the number of substance P receptors in rheumatoid synovium is increased compared with normal synovium. It is now widely accepted that there is a neurogenic mechanism in the pathophysiology of arthritis, and substance P may be one of the important mediators. Receptor binding sites for substance P are expressed at high concentrations in arterioles, venules and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease.

In Chapter 8.1 the potential usefulness of a new radiolabelled substance P analogue, [^{111}In-DTPA-Arg^1]-substance P, as a radiopharmaceutical for the in vivo detection of substance P receptor positive immunological disorders and tumours is evaluated. Substance P, [3-(p-hydroxyphenyl)propionyl-Arg ^{1}]-substance P (Bolton-Hunter-substance P, BH-substance P), and [DTPA-Arg^1]-substance P, inhibited dose-dependently the binding of [^{125}I]-BH-substance P to the substance P receptors in rat brain cortex membranes with IC_{50} values of 0.2, 2 and 4 nM, respectively. In an autoradiographic displacement study of the submandibular gland with [^{125}I]-BH-substance P as radiopharmaceutical, an IC_{50} of 2.7 nM was found for [DTPA-Arg^1]-substance P. In vivo metabolism of the radiopharmaceutical in the rat revealed a renal clearance of 50% of the injected radioactive dose in 30 minutes, and a rapid enzymatic degradation of the radiopharmaceutical, resulting in an effective half-life of the radiopharmaceutical in blood of approximately 3 minutes. Four and 24 hours after the injection of [^{111}In-DTPA-Arg^1]-substance P, uptake in and specific binding to substance P receptor positive organs and tumours were found, with a (substance P receptor positive) target to background optimum at 24 hours. Tissue distribution and ex vivo autoradiographic studies in rats, with and without pretreatment with the selective non-peptide neurokinin 1 receptor antagonist CP96,345, showed uptake and specific binding of radioactivity in isolated tumours, and in the

submandibular and parotid glands. Visualization of normal substance P receptor positive tissues such as the salivary glands by gamma camera scintigraphy, after administration of [^{111}In-DTPA-Arg^1]-substance P, was demonstrated in untreated rats. Pathological substance P receptor processes were visualized in rats bearing the transplantable pancreatic tumour CA20948, and in rats with adjuvant arthritis, induced by injection of a homogenate of *Mycobacterium tuberculosis*.

In Chapter 8.2 we present our results concerning the metabolism of the substance P analogue [^{111}In-DTPA-Arg^1]-substance P in man, as well as the visualization of the thymus in patients with immune mediated diseases. Twelve selected patients, five with inflammatory bowel disease, one with ophthalmic Graves' disease, one with sarcoidosis, one with Sjögren's syndrome, one with rheumatoid arthritis, two with myasthenia gravis, and one with systemic lupus erythematosus were investigated. During and after intravenous administration of 200 MBq (2.5 μg) [^{111}In-DTPA-Arg^1]-substance P, blood pressure, heart rate, and oxygen saturation were monitored. Radioactivity was measured in blood, urine and faeces during 48 hours after injection. Planar and single photon emission tomography (SPECT) images were obtained 4 and 24 hours after injection. After administration of [^{111}In-DTPA-Arg^1]-substance P a transient flush was observed in all patients. Degradation of [^{111}In-DTPA-Arg^1]-substance P started in the first minutes after administration, resulting in a half-life of 10 minutes for the total plasma radioactivity, and of 4 minutes for the intact radiopharmaceutical, as identified with high performance liquid chromatography. Urinary excretion amounted in >95% of the radioactivity within 24 hours after injection, and up to 0.05% was found in the faeces up to 60 hours. In all patients uptake of radioactivity was found in the parotid glands, areolae of the mammae (in women), liver, kidneys, and urinary bladder. In eight patients a high uptake of [^{111}In-DTPA-Arg^1]-substance P was observed in the thymus. These data indicate that, despite its short half-life, [^{111}In-DTPA-Arg^1]-substance P, a new radiopharmaceutical, can be used to visualize the thymus. This may contribute to the investigation of the role of the thymus in immune related diseases.

In Chapter 9 the applications of somatostatin and substance P are summarized with respect to their use as radioimagers. The number of neuropeptide receptors are upregulated in autoimmune diseases and haematological malignancies. The immune modulating properties of these neuropeptides may warrant speculations

on the possible role of neuropeptide receptors as a new target in the treatment of these diseases in the near future. Neuropeptide scintigraphy will be important in the determination of the neuropeptide receptor status, which may be related to the efficacy of therapy.

SAMENVATTING

Dit proefschrift beschrijft de resultaten van onderzoek naar de expressie van somatostatine en substance P receptoren bij autoimmuun ziekten en maligne lymfomen. In dit onderzoek zijn verschillende onderzoekstechnieken toegepast. Voor het in vitro onderzoek werd gebruik gemaakt van peptide receptor autoradiografie, ligand binding studies en polymerase ketting reacties. In vivo onderzoek werd verricht met behulp van peptide receptor scintigrafie.

In Hoofdstuk 1 wordt de biologie van somatostatine besproken. Somatostatine is een klein peptide, bestaat uit 14 aminozuren en komt in vrijwel het gehele lichaam voor. De hoogste concentraties somatostatine worden gevonden in het centraal zenuwstelsel en in de endocriene klieren. Somatostatine remt de afgifte van bepaalde hormonen, zoals groeihormoon. Van deze eigenschap wordt gebruik gemaakt bij de behandeling van endocrien actieve tumoren. Behandeling met een somatostatine analogon, zoals octreotide, resulteert in een vermindering van de hormoon afgifte door deze tumoren. De somatostatine receptor heeft zeven transmembraan domeinen en is gebonden aan een G eiwit. Momenteel zijn vijf receptor subtypen bekend. De voor therapeutische doeleinden beschikbare somatostatine analoga, zoals octreotide en BIM-23014, binden vooral aan receptor subtypen 2 en 5.

Een andere toepassing van somatostatine analoga is het afbeelden van ziekteprocessen door middel van peptide receptor scintigrafie. Door binding van een radionuclide (^{111}Indium) aan octreotide, via een DTPA groep, ontstaat een radioimager waarvan is aangetoond dat deze zeer goed bruikbaar is voor de detectie van endocriene tumoren. Laatst genoemde tumoren hebben een hoge dichtheid aan somatostatine receptoren.

In Hoofdstuk 2 wordt de literatuur samengevat omtrent de relatie tussen somatostatine en het immuun systeem. Somatostatine receptoren komen voor op geactiveerde lymfocyten en monocyten. Somatostatine heeft een overwegend remmende werking op het immuunsysteem. De produktie van immuunglobulines (vooral IgA), de proliferatie van lymfocyten, de afgifte van cytokines, zoals interferon γ en tumor necrosis factor α, en de afgifte van histamine door mestcellen worden geremd. Verschillende onderzoeken hebben aangetoond dat somatostatine geproduceerd kan worden in granulomateuze ontstekingsprocessen, wat betekent dat somatostatin

mogelijk een autocrien of paracrien regulerende functie heeft. Somatostatine wordt bovendien uitgescheiden door zenuw vezels, hetgeen een interactie tussen het zenuwstelsel en het immuun apparaat suggereert.

In Hoofdstuk 3 worden de resultaten beschreven van autoradiografisch onderzoek naar de expressie van somatostatine receptoren in vitro op weefsel coupes van patiënten met maligne lymfomen. In de groep non-Hodgkin lymfomen bleek 91% van de laaggradige lymfomen, 100% van de lymfomen van intermediaire maligniteitsgraad, en 70% van de hooggradige lymfomen receptoren voor somatostatine tot expressie te brengen. Deze receptoren hebben een hoge affiniteit voor het somatostatine analogon [Tyr^3-octreotide]. Met behulp van somatostatine receptor scintigrafie werden tumor lokalisaties zichtbaar gemaakt bij patiënten met de ziekte van Hodgkin of een non-Hodgkin lymfoom. Met deze techniek werden meer tumor lokalisaties gevonden dan met conventionele stageringsmethoden, hetgeen bij een aantal patiënten leidde tot een verandering van het ziektestadium. Dit laatste kan gevolgen hebben voor de te volgen therapie. Recent werd aangetoond dat octreotide behandeling een gunstig effect heeft op lymfomen met een lage maligniteitsgraad. Expressie van somatostatine receptoren biedt in de toekomst wellicht mogelijkheden voor de toepassing van somatostatine analoga, gebonden aan een ß stralend radionuclide, bij de bestraling van tumoren.

Hoofdstuk 4 en 5 beschrijven onderzoek naar het voorkomen van somatostatine receptoren bij granulomateuze ontstekingen, reumatoide artritis en de ziekte van Sjögren. In vitro receptor autoradiografie toonde specifieke hoge affiniteits receptoren aan in granulomen in de epitheloid cel regio en in de hoog endotheliale venulen in synovia van patiënten met reumatoide artritis. Met behulp van somatostatine receptor scintigrafie was het mogelijk ontstekingslokalisaties en ontstoken gewrichten bij vrijwel alle patiënten af te beelden en aldus uitbreiding van de ziekte te objectiveren. De expressie van somatostatine receptoren is sterk gerelateerd aan de activiteit van deze ziekten. Bij sarcoïdose toonde het scintigram bij patiënten die goed reageerden op therapie geen afwijkingen meer, terwijl bij patiënten die niet goed reageerden op therapie het scintigram onveranderd, dus afwijkend, bleef. Ook bij patiënten met reumatoide artritis correleerden de klinische symptomen (pijn en zwelling van de gewrichten) goed met de opname van radioactiviteit in de gewrichten.

Hoofdstuk 6 beschrijft het onderzoek naar de expressie van somatostatine receptor subtypen in lymfatische cellijnen met behulp van een polymerase ketting reactie. Vier van de 18 onderzochte cellijnen brachten messenger RNA coderend voor somatostatine receptor subtype 2 tot expressie. Ligand binding studies toonden aan dat dit bij drie cellijnen leidde tot expressie van membraan receptoren.

In Hoofdstuk 7 wordt de relatie beschreven tussen substance P en het immuun systeem. Substance P komt vooral voor in het centraal zenuwstelsel, alwaar het als neurotransmitter een rol speelt in de nociceptieve signaal transmissie. In tegenstelling tot de remmende werking van somatostatine, heeft substance P een activerende invloed op het immuun systeem, zoals stimulatie van de synthese van immuunglobulines, de afgifte van histamine door mestcellen, en de afgifte van interleukine 1, interleukine 6 en tumor necrosis factor α door monocyten. Upregulatie van substance P receptoren wordt gevonden bij een aantal immuungemedieerde ziekten, onder andere bij reumatoide artritis in de aangedane synovia, bij inflammatory bowel disease en ook bij zenuw laesies waar substance P receptoren tot expressie worden gebracht door glia cellen. Substance P en somatostatine lijken ten aanzien van het immuun systeem een tegengestelde werking te hebben.

In Hoofdstuk 8 worden het metabolisme en de bindingskarakteristieken beschreven van de nieuwe radioimager [^{111}In-DTPA-Arg^{1}]-substance P. Deze radioimager heeft een hoge affiniteit voor submandibulaire klieren en hersen cortex membranen in de rat. Ondanks de korte halfwaarde tijd kan deze stof gebruikt worden voor het afbeelden van substance P receptor positieve processen. In dierexperimenten (rat) konden adjuvant artritis en transplantabele tumoren worden afgebeeld. Bij de mens werd, door middel van substance P receptor scintigrafie, de thymus zichtbaar gemaakt bij patiënten met autoimmuun ziekten. Tevens konden ontstekingsprocessen zichtbaar worden gemaakt bij patiënten met ophtalmic' Graves disease, inflammatory bowel disease en de ziekte van Sjögren.

In Hoofdstuk 9 wordt de mogelijke rol besproken van somatostatine en substance P receptor expressie in de behandeling van maligne lymfomen en autoimmuun processen.

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CURRICULUM VITAE

The author of this thesis was born on the 11th of October 1958 in Schiedam. In 1979 he finished the training for laboratory technician in clinical chemistry at the 'van Leeuwenhoek Instituut' in Delft. He attended medical school at the Erasmus University Rotterdam (1980-1987). During his medical study he worked as a laboratory technician at the department of Haematology of the University Hospital Rotterdam (Prof. Dr. J. Abels and Dr. C. van der Heul). In 1987 he worked for two months as a student in the Tjikini Hospital in Jakarta, Indonesia. In 1988 he started his training in internal medicine at the department of Internal Medicine III of the University Hospital Rotterdam (Prof. Dr. J.C. Birkenhäger). He was registered as specialist for internal diseases in September 1993. Since October 1993 he works at the departments of Immunology (Prof. Dr. R. Benner) and Internal Medicine III (Prof. Dr. J.C. Birkenhäger) of the University Hospital Rotterdam.

List of publications

Bakker WH, Krenning EP, Reubi JC, Breeman WAP, Setyono-Han B, de Jong M, Kooij PPM, Bruns C, van Hagen PM, Marbach P, Visser TJ, Pless J, Lamberts SWJ. In vivo application of [^{111}In-DTPA-D-Phe^{1}]-octreotide for detection of somatostatin receptor-positive tumors in rats. Life Sci 1991;49:1593-601.

Reubi JC, Waser B, van Hagen PM, Lamberts SWJ, Krenning EP, Gebbers JO, Laissue JA. In vitro and in vivo detection of somatostatin receptors in human malignant lymphomas. Int J Cancer 1992;50:895-900.

Krenning EP, Bakker WH, Kooij PPM, Breeman WAP, Oei HY, de Jong M, Reubi JC, Visser TJ, Bruns C, Kwekkeboom DJ, Reijs AEM, van Hagen PM, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe ^{1}]-octreotide in man: metabolism, dosimetry and comparison with [^{123}I-Tyr^{3}]-octreotide. J Nucl Med 1992;33:652-8.

Krenning EP, Kwekkeboom DJ, Reubi JC, van Hagen PM, van Eijck CH, Oei HY, Lamberts SWJ. ^{111}In-octreotide scintigraphy in oncology. [Review] Metabolism 1992;41 Suppl 2:83-6.

Krenning EP, Kwekkeboom DJ, Oei HY, Reubi JC, van Hagen PM, Kooij PPM, Reijs AEM, Lamberts SWJ. Somatostatin receptor imaging of endocrine gastrointestinal tumors. Schweiz Med Wochenschr 1992;122:634-7.

van Hagen PM, Krenning EP, Reubi JC, Mulder AH, Bakker WH, Oei HY, Löwenberg B, Lamberts SWJ. Somatostatin analogue scintigraphy of malignant lymphomas. Br J Haematol 1993;83:75-9.

Krenning EP, Kwekkeboom DJ, Reubi JC, van Hagen PM, van Eijck CH, Oei HY, Lamberts SWJ. ^{111}In-octreotide scintigraphy in oncology. Digestion 1993;54 Suppl 1:84-7.

Krenning EP, Kwekkeboom DJ, Bakker WH, Breeman WAP, Kooij PPM, Oei HY, van Hagen PM, Postema PTE, de Jong M, Reubi JC, Visser TJ, Reijs AEM, Hofland LJ, Koper JW, Lamberts SWJ. Somatostatin receptor scintigraphy with [^{111}In-DTPA-D-Phe^{1}]- and [^{123}I-Tyr^{3}]-octreotide: the Rotterdam experience with more than 1000 patients. [Review] Eur J Nucl Med 1993;20:716-31.

van Hagen PM, Markusse HM, Lamberts SWJ, Kwekkeboom DJ, Reubi JC, Krenning EP. Somatostatin receptor imaging. The presence of somatostatin receptors in rheumatoid arthritis. Arthritis Rheum 1994;37:1521-7.

van Hagen PM, Krenning EP, Kwekkeboom DJ, Reubi JC, van den Anker-Lugtenburg PJ, Löwenberg B, Lamberts SWJ. Somatostatin and the immune and haematopoietic system; a review. [Review] Eur J Clin Invest 1994;24:91-9.

van Hagen PM, Krenning EP, Reubi JC, Kwekkeboom DJ, Bakker WH, Mulder AH, Laissue JA, Hoogsteden HC, Lamberts SWJ. Somatostatin analogue scintigraphy in granulomatous diseases. Eur J Nucl Med 1994;21:497-502.

Reubi JC, Waser B, Markusse HM, Krenning EP, van Hagen PM, Laissue JA. Vascular somatostatin receptors in synovium from patients with rheumatoid arthritis. Eur J Pharmacol 1994;271:371-8.

van den Anker-Lugtenburg PJ, Krenning EP, Oei HY, Gerrits CJH, van Hagen PM, Reubi JC, Lamberts SWJ, Löwenberg B. The role of somatostatin receptor scintigraphy (SRS) in the initial staging of malignant lymphoma. [Abstract] J Nucl Med 1994;35:132.

Feron EJ, Rothova A, van Hagen PM, Baarsma GS, Suttorp-Schulten MS. Interferon-alpha 2b for refractory ocular Behçet's disease [letter]. Lancet 1994;343:1428.

Jansen A, van Hagen PM, Drexhage HA. Defective maturation and function of antigen-presenting cells in type 1 diabetes. Lancet 1995;345:491-2.

Bac DJ, van Hagen PM, Postema PTE, ten Bokum AM, Zondervan PE, van Blankenstein M. Octreotide for protein-losing enteropathy with intestinal lymphangiectasia. Lancet 1995;345:1639.

van Hagen PM, de Leeuw K, Hagemeijer A, Löwenberg B. Phagocytic plasma cells in a patient with multiple myeloma. Neth J Med 1995;46:25-9.

van Hagen PM, van den Anker-Lugtenburg PJ, Reubi JC, Lamberts SWJ, Krenning EP. The role of octreotide scintigraphy in malignant lymphomas, granulomatous diseases and rheumatoid arthritis. Handbook of targeted delivery of imaging agents. Torchilin VP, editor. CRC Press, Inc., 1995:631-44.

Heule F, van Hagen PM, van Dongen JJM, Krenning EP, Reubi JC, van Joost T. Staging of Sézary syndrome with somatostatin receptor scintigraphy. Br J Dermatol, in press.

Kooij A, de Heide LJM, Engelkens HJH, Mulder AH, van Hagen PM, Schalm SW. How to diagnose autoimmune hepatitis in systemic lupus erythematosus? Hepatology, in press.

Breeman WAP, van Hagen PM, Visser-Wisselaar HA, van der Pluijm ME, Koper JW, Setyono-Han B, Bakker WH, Kwekkeboom DJ, Lamberts SWJ, Visser TJ, Krenning EP. Substance P receptor scintigraphy: initial studies in vitro and in rats. J Nucl Med, in press.

Kooij A, de Heide LJM, Engelkens HJH, Mulder AH, van Hagen PM, Schalm SW. Hepatitis in a patient with systemic lupus erythematosus: Is is autoimmune hepatitis? Neth J Med, in press.

van den Anker-Lugtenburg PJ, Heule F, van Hagen PM, van Joost Th, Oei HY, Löwenberg B, Krenning EP. Somatostatin receptor scintigraphy in cutaneous malignant lymphomas. Submitted for publication.

van den Anker-Lugtenburg PJ, Krenning EP, Oei HY, van Hagen PM, Gerrits CJH, Reubi JC, Lamberts SWJ, Löwenberg B. Somatostatin receptor scintigraphy in the initial staging of Hodgkin's disease. Submitted for publication.

van Hagen PM, Breeman WAP, Reubi JC, Postema PTE, van den Anker-Lugtenburg PJ, Robers C, Lamberts SWJ, Kwekkeboom DJ, Visser TJ, Krenning EP. Thymus scintigraphy with [^{111}In-DTPA-Arg^1]-substance P. Submitted for publication.

van Hagen PM, Lichtenauer-Kaligis EGR, ten Bokum AMC, Visser-Wisselaar HA, Hofland LJ, Hazenberg MP, van Dongen JJM, Lamberts SWJ. Somatostatin receptor subtype expression in human lymphoid cell lines. Submitted for publication.

Kooij A, van Hagen PM, Schalm SW. Hepatic involvement in systemic lupus erythematosus. Submitted for publication.