

Novel Therapeutic Targets for Somatostatin in Inflammatory Chronic Diseases

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Abstract: Somatostatin binds to five receptors sst1-sst5, belonging to the G-protein coupled receptor super family. So far, only sst2 preferring analogs, presenting also high affinity for sst5 and moderate affinity for sst3, are available for clinical use to treat certain hormonal disorders and tumors (pituitary adenomas and gastroenteropancreatic tumors) with long-lasting efficacy and minimal side-effects as observed in patients with acromegaly. Recent strategies based on sequence modifications, such as D-substitutions, deletions, backbone cyclisation technology, novel thiourea scaffolds, along with combinatorial chemistry, lead to the discovery of peptide and non peptide compounds, with either combined affinities for two or more receptor subtypes, or exclusive selectivity for one of them, or a universal profile binding, more stable than the natural peptides. A large field of potential novel drugs has been open. Molecular mechanisms for anti-inflammatory properties of somatostatin and analogs involve anti-secretory, anti-proliferative and anti-angiogenic properties, which may be receptor selective. The great diversity of new analogs and major progress in the understanding of biological activity of somatostatin and receptors support strategies for targeting somatostatin to treat some chronic inflammatory diseases which are still a major cause of disability.

Keywords: Somatostatin, somatostatin receptors, somatostatin analogs, chronic inflammatory diseases, cytokines, apoptosis, inhibition of cell proliferation, angiogenesis.

1. INTRODUCTION

The role of somatostatin in inflammatory chronic diseases has been proposed in Graves ophtalmopathy, granulomas and rheumatoid arthritis and evaluated in a number of experimental models. This role is largely supported by the characterization of somatostatin and somatostatin receptors (sst_n) in cells from inflamed organs and cells of the immune system. However, the clinical use of somatostatin is limited to the treatment of acromegaly and neuroendocrine tumors by sst2 analogs.

Recent progress in the somatostatin field provided us with a series of new analogs of somatostatin, agonists or antagonists. New transduction pathways triggered by somatostatin have now been discovered. Consequently, there is a better understanding of the molecular mechanisms whereby somatostatin modulates the immune response and controls inflammation. It is suggested that somatostatin may play a fundamental role in the regulation of inflammatory process acting both on the epithelial cell targets and immune cells source of immune agents. Targeting somatostatin receptors with agonists and antagonists for therapeutic purposes and drug discovery in inflammatory diseases could be rewarding. Therefore, it is timely to reevaluate the role of somatostatin in inflammation.

After a brief description of somatostatin and sst_n structure, strategies to discover new somatostatin analogs and structure-activity relationships will be described. The

clinical, experimental and molecular aspects relevant to the functional role of somatostatin in inflammatory diseases will be presented.

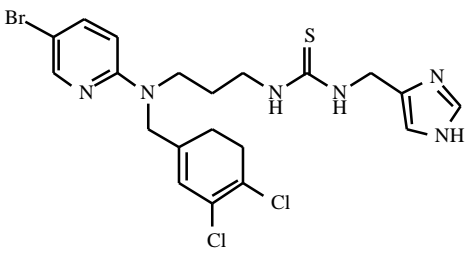
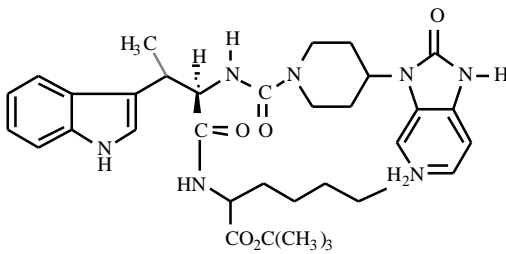
2. SOMATOSTATIN AND SOMATOSTATIN RECEPTORS

Somatostatin has been isolated under two forms : a 14 amino-acid peptide, somatostatin-14, isolated from the hypothalamus in 1973 [1], and a 28 amino-acid residue peptide somatostatin-28, isolated in 1980 from the pig intestine [2]. These two forms derive from a single precursor protein. Somatostatin-28 is a N-terminal extension of somatostatin-14, this extension being completely inactive by itself (Table I).

The biological activity of somatostatin is generally inhibitory. It has been first described as an inhibitor of GH (Growth Hormone) release and called SRIF (Somatotropin Releasing Inhibitory Factor). The ubiquitous distribution of the peptide has been the driving force for the discovery of important inhibitory functions in pituitary, exocrine and endocrine pancreas, gastro-intestinal tract and neural transmission. Somatostatin acts as an inhibitor of hormone release : along with the inhibition of growth hormone release, it inhibits the release of Thyroid-Stimulating Hormone (TSH), Thyrotropin-Releasing Hormone (TRH), Corticotropin-Releasing Hormone (CRH), insulin, glucagon, gastrin, cholecystokinin, and Vaso-Intestinal Peptide (VIP). Moreover, it is an inhibitor of exocrine gastro-intestinal and pancreatic secretions. It modulates neurotransmission, intestinal motility, absorption of nutrients and vascular contractility. Rapidly, a large number of diversified cellular targets have been characterized, including inflammatory

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Table 1. Chemical Characterization of Somatostatin Natural Peptides, Somatostatin-like Peptides, Peptide Analogs and Non-Peptide Analogs

Somatostatin-14 [1]	Ala ₁ -Gly ₂ -c(Cys ₃ -Lys ₄ -Asn ₅ -Phe ₆ -Phe ₇ -Trp ₈ -Lys ₉ -Thr ₁₀ -Phe ₁₁ -Thr ₁₂ -Ser ₁₃ -Cys ₁₄)
Somatostatin-28 [2]	Ser ₁ -Ala ₂ -Asn ₃ -Ser ₄ -Asn ₅ -Pro ₆ -Ala ₇ -Met ₈ -Ala ₉ -Pro ₁₀ -Arg ₁₁ -Glu ₁₂ -Arg ₁₃ -Lys ₁₄ -somatostatin-14
Cortistatin 14 [5]	Pro (Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys)-Lys
Cortistatin 17 [6]	Asp-Arg-Met-Pro (Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys)-Lys
L-363,301 [9]	c(Pro-Phe-DTrp-Lys-Thr-Phe)
SMS 201-995 [10]	DPhe-c(Cys-Phe-DTrp-Lys-Thr-Cys)-Thr-ol
RC160 [11]	DPhe-c(Cys-Tyr-DTrp-Lys-Val-Cys)-Trp-NH ₂
BIM23014 [9]	DNaI c(Cys-Tyr-DTrp-Lys-Val-Cys)-Thr-NH ₂
TT-232 [12]	DPhe-c(Cys-Tyr-DTrp-Lys-Cys)-Thr-NH ₂
PTR 3173 [15]	c(GABA-Phe-Trp-DTrp-Lys-Thr-Phe-GlyC3-NH ₂)
CH-275 [17]	Des-AA _{1,2,5} [D-Trp, N-p-isopropyl-4-aminomethyl-L-Phe]-somatostatin-14
sst3-ODN8 [19]	carbamoyl-des-AA _{1,2,4,5,12,13} [D-Cys,Tyr,D-Agl(Me,2-naphthoyl)]-somatostatin-14
PTR 3046 [21]	c[PheN2-Tyr-DTrp-Lys-Val-PheC3]-Thr-NH ₂
KE108 [23]	Tyr-c (DDab-Arg-Phe-Phe-DTrp-Lys-Thr-Phe)
SOM230 [24]	c[(diaminoethylcarboethylcarbamoyl)HyPro-Phg-DTrp-Lys-Tyr(Bzl)-Phe]
NNC 26-9100 [20]	
L 054-522 [18]	

cells. The unique specificity devoted to somatostatin-28 is an increase affinity to inhibit the release of insulin.

The biological activity of somatostatin is due to the binding of the peptide to specific receptors. Five *sst_s* have been sequenced and cloned in the 1990s, so-called *sst1-sst5* [1]. They are seven transmembrane domains G-protein coupled receptors (GPCR) possessing strong structural similarities. They are encoded by 5 separate genes, located on 5 different chromosomes, intronless except for one of them, the *sst2* receptor. Two isoforms of *sst2* exist : a long

variant composed of 369 amino-acids (*sst2A*) and a shorter variant lacking 23 residues (*sst2B*), observed mainly in rat and mouse.

Since somatostatin receptors have not been crystallized, their tridimensional structure is based on the rhodopsin model. Like other GPCRs, all *sst_s* are monomeric transmembrane proteins, with N-terminal domain outside the cell, and C-terminal fragment inside the cell. The central core of the protein is composed of seven trans-membrane hydrophobic domains linked by three extracellular loops and

three intracellular loops. The N-terminal domain and the second extracellular loop possess single or several N-linked glycosylation sites. A disulphide bridge links two cysteines in the 1st and 2nd extracellular loops. The transmembrane domains have the greatest amino-acid similarity. The three intracellular loops and the C-terminal domain possess phosphorylation sites for protein kinase A and protein kinase C. A cysteine located 12 amino-acids downstream from the 7th transmembrane domain, in the C-terminal fragment, is a probable site of palmitoyl membrane anchor.

The ligand binding pocket for natural peptides, somatostatin-14 and 28 has been determined indirectly. It is composed of several residues in the 2nd extracellular loop and transmembrane domains 3 to 7 exclusively. These residues are crucial to interact with residues of the ligand, stabilizing the conformation of the receptor. Gln₂₉₁ and Ser₃₀₅ have been identified in sst1, and Asn₂₇₆ and Phe₂₉₄ in sst2, to interact with the Phe₇, Trp₈, Lys₉ and Thr₁₀ residues of somatostatin. As flexible peptides, somatostatin-14 and somatostatin-28 interact with any type of receptors, almost equally in the nanomolar range.

Sst_s are coupled to G-proteins. The nature of the G-protein(s) to which they are coupled, could not be deduced from the amino-acid sequence of the intracellular domains. Based on biological studies, G_{i/o} and/or G₁₃ have been suspected to couple to sst_s. By contrast, it has recently been possible to identify specific amino-acid motifs in sst_s that confer a selective effector activation. Sst1, sst3 and sst4, but not sst2 and sst5, possess a T/S/P-V motif in the 2nd intracellular domain and a Q-Q/R motif in the 3rd intracellular domain, responsible for coupling to Na⁺-H⁺ Exchanger type 1 (NHE1) [3]. Also, two Immunoreceptor Tyrosine-based Inhibitory Motifs (ITIMs) have been identified within the 3rd intracellular domain (L-C-Y₂₂₈-L-F-I) and the C-terminal part (I-L-Y₃₁₂-A-F-L) of sst2 [4]. ITIMs are composed of a consensus sequence of amino-acids (I/V/L/S-x-Y-x-x-L/V/I/S) including a tyrosine. Upon the phosphorylation of this tyrosine, ITIM-bearing proteins recruit SH2 containing phosphotyrosine phosphatases (PTP_s), leading to enzyme activation and consequently to the inhibition of phosphorylation-dependent signaling cascades. Therefore, selective recruitment of signaling proteins to the intracellular domains of somatostatin receptors could directly activate the signaling pathways.

In 1996, a gene encoding for a peptide so-called **cortistatin** has been cloned and characterized from the cerebral cortex and the hippocampus [5]. This peptide share 11 of 14 amino-acid residues of somatostatin, including the two cysteines necessary for cyclization. However, it is the product of a gene different from the somatostatin gene. Since then, cortistatin peptides have been discovered in human [6]. They are three 14, 17 and 29 amino-acid forms of peptides, possessing strong structural similarities with somatostatin. They bind to all sst_s, sst1, sst3 and sst4 with a higher affinity than somatostatin, and sst2 and sst5 with a 100-fold lower affinity than somatostatin.

The demonstration of novel specific biological activities of this peptide is a very interesting finding. It has been suggested to be the endogenous ligand for somatostatin

receptors in the human immune system [7]. Very recently, an orphan receptor MrgX2 of the Mrg (mas related genes) family has been characterized as a high potency cortistatin receptor [8].

3. SOMATOSTATIN AGONISTS AND ANTAGONISTS : STRUCTURE-ACTIVITY RELATIONSHIPS (TABLE 1 AND 2)

The most important characteristics of the natural somatostatin peptides are : 1) instability : a very short half-life (1-3 minutes in human plasma) is preventing their clinical use. 2) non-selectivity : they bind to all sst_s with almost the same very high affinity, except somatostatin-28, which binds to sst5 with a higher affinity than somatostatin-14. Therefore, great efforts are currently made to find out more selective and/or more stable analogs [9,10].

Two main approaches are used. The first one consists in modifications introduced in the amino-acid sequence of somatostatin to improve the peptide stability : deletions, D-substitutions, or replacement of somatostatin backbone by diverse non-peptide compounds forming scaffolds. Deletions that cause no loss of potency, are located in the amino-terminal part of the peptide. In the minimum active form to inhibit GH secretion, the residues Phe₇, Trp₈, Lys₉, Thr₁₀, have been shown to be crucial for interaction with the receptors and biological activity of the peptide. The replacement of L-form amino-acids by the D-form increases peptide stability, due to greater resistance against degradation or to stabilization in a more active conformation. Therefore, the substitution of Trp₈ by DTrp₈ is among the most frequent modifications. DTrp₈ somatostatin has a higher potency than the natural peptide and is more stable. Also, it was demonstrated that the role of the peptide backbone was to support the side chains of Phe₇, Trp₈, Lys₉, Thr₁₀ in the right spatial conformation. As suggested by molecular modeling studies, non-peptide scaffolds, such as carbohydrates or thiourea, were used to mimic side-chain conformation and present the correct chemical groups in the right position to obtain compounds with high affinity and selectivity.

Combinatorial chemistry associated with high throughput receptor binding techniques is another, novel and very productive approach. Hundreds of thousands of new chemical entities, non peptide analogs, could be examined in a short period of time. Lead structures were selected from the Merck collection based on "privileged structure" observed in somatostatin peptides. Essential amino-acid side-chains, in modeled hexapeptide minimal structure served as a probe. Also, libraries were based on backbone cyclization technology, by bridging any two positions along the backbone by different bonds in size and chemical composition.

These different approaches led to the discovery of three basic classes of compounds, that are most important on a pharmacological point of view : 1) compounds with combined high affinities for two or more sst_s; 2) compounds with exclusive selectivity for one of the five sst_s, and 3) compounds with a rather universal binding profile that mimic the profile of natural somatostatin by binding to all of the five sst_s with high affinity.

Table 2. Binding Affinity of Natural Peptides of Somatostatin, Somatostatin-like Peptides, Peptide Analogs and Non-Peptide Analogs for Cloned Human Somatostatin Receptors. Assays were Performed with Membranes Isolated from Transfected Cells. Values (nM) are Ki or IC50(*) Calculated from Each Competition Curves in Ligand Binding Assays. Data are Derived from Reference given in Brackets. Data from TT-232 are not available for Cloned Human Receptors

	sst1	sst2	sst3	sst4	sst5
Somatostatin-14 [1]	0.1-2.26	0.2-1.3	0.3-1.6	0.3-1.8	0.2-0.9
Somatostatin-28 [1]	0.1-2.2	0.2-4.1	0.3-6.1	0.3-7.9	0.05-0.4
Cortistatin 14 [5]	2.1±0.8	0.5±0.1	3.8±0.9	18.2±2.5	0.9±0.2
Cortistatin 17 [6] *	7	0.6	0.6	0.5	0.4
L-363,301[9]	>1,000	5.1	129	>1,000	25
Octreotide or SMS 201-995 [1]	>1,000	0.4-2.1	4.4-34.5	>1,000	5.6-32
Vapreotide or RC160 [1]	>1,000	5.4	31	45	0.7
Lanreotide or BIM23014 [1]	>1,000	0.5-1.8	43-107	66-2100	0.6-14
PTR 3173 [15]*	>1,000	3±0.75	>100	7±1.1	1±0.05
CH-275 [17]*	30.9±13	>10,000	345±195	>1,000	10,000
Sst3-ODN8 [19]*	>10,000	>10,000	6.7±2.6	>10,000	>10,000
NNC 26-9100 [20]	1800	621	1400	6	1,900
KE 108 [23]*	2.6±0.4	0.9±0.1	1.5±0.2	1.6±0.1	0.65±0.1
SOM230 [24]	8.2	9.0	9.1	<7.0	9.9
L-054,522 [18]	2.4	0.01	31	81	163
PTR 3046 [21]*	>1,000	>1,000	>1,000	>1,000	67

3.1 Somatostatin Analogs with Combined High Affinities for Several Receptors

The minimum active form of somatostatin-14 to inhibit GH secretion contains the 7-10 amino-acid fragment of somatostatin. It is about 0.02% as potent as the natural peptide. Therefore, a series of analogs have been synthesized, containing the sequence Phe-DTrp-Lys-Thr. The most active is the **L-363,301** compound [9], forming a cyclic hexapeptide compound, with an amid linkage between Pro and Phe. The bridging region (Phe-Pro) has been considered to be necessary for high activity. Its role is to stabilize a II' turn around the Phe-DTrp-Lys-Thr and maintain the right orientation of the amino-acid side-chains for the bioactive conformation of somatostatin.

Then, another series of analogs was elaborated from the L-363,301 compound, incorporating basic or acidic peptoid residues. The presence of an acidic residue in the bridging region prevents the binding of somatostatin to sst5, whereas the presence of a basic residue seems to be helpful. These analogs did not bind to sst1 receptors. The most important analogs, for clinical use, potency and stability, are found in this series [10].

Octreotide, so-called **SMS 201-995**, or **sandostatin**, is an octapeptide, with the D-isofom of Trp₈, the D-isofom of Phe₁ at the N-terminal end of the peptide, protecting the disulfide bridge against enzymatic degradation, and a Thr-ol

at the C-terminal end. It binds to sst2, and sst5 receptors with a very high affinity, and to sst3 with a moderate affinity, whereas it binds to sst1 and sst4 receptors with a very low affinity. It inhibits selectively GH release. It is more stable and has a higher potency than the natural peptide. A number of analogs were synthesized, based on octreotide. Among them, RC160 and BIM 23014 are the most widely used.

RC160 or **vapreotide** : like octreotide, this analog is more selective for sst2, sst5, than sst3. It provides a much higher activity than its parent compound and has been especially examined to determine the mechanism of action of somatostatin in inhibiting cancer growth [11].

BIM 23014 or **lanreotide** : this analog is more selective for sst5 than for sst2 and sst3 [9].

TT-232 : A series of new analogs was synthesized to discriminate endocrine and antitumor effects. Substitutions were carried out in all positions except for Lys in position 10. The most active of these compounds, is an heptapeptide with a cyclopenta-ring structure analog of somatostatin, TT-232. TT-232 was found to be devoided of GH release inhibitory activity but to possess strong antiproliferative effects. It binds with a high affinity to sst1 and sst4. Also, this compound was found to inhibit inflammation in a number of experimental models [12,13].

Recently, novel analogs were synthesized by using the backbone cyclization technology, constructing a lactam

bridge for cyclization of a minimum active form of somatostatin. The lead compound, **PTR 3173** possesses *in vivo* endocrine complete selectivity for the inhibition of GH release with no effect on glucagon or insulin release [14,15]. It binds with a high affinity to sst2, sst4 and sst5. Analogs of PTR 3173, in which the lactam bridge was replaced by a backbone disulfide bridge, retain metabolic stability but receptor binding specificity is affected. With these compounds, the idea that biological selectivity might involve pharmacological non-selectivity emerged for the first time.

3.2 Selective Receptor Subtype Somatostatin Analogs

Classical chemistry succeeded in designing new stable and selective peptide compounds. Also, the development of somatostatin analogs with very high selectivity and affinity for the different receptor subtypes came from combinatorial strategy and are non-peptide agonists. These compounds are thought to be useful for pathophysiological studies to discover the function of each receptor and consequent clinical use [16].

A family of analogs was designed by introducing deletions in the N-terminal part of somatostatin, DTrp₈ substitution, and replacement of Lys in position 9 by N-p-isopropyl-4-aminomethyl-L-phenylalanine (IAmp) [17]. The basicity of the terminal nitrogen in IAmp suggested it could replace easily Lys₉. **CH-275** is the lead compound of this family. It has a higher affinity and selectivity for sst1 (Table 1). The iodinated analog DesAA_{1,2,5} [DTrp₈, IAmp₉] Tyr₁₁ somatostatin labelled only sst1 expressing cells and identified sst1 expressing tissues using *in vitro* autoradiography.

Postponed for years, since the first agonists sandostatin, vapreotide, and lanreotide were stable peptides and bound sst2 with a high affinity, a family of new potent and selective sst2 agonists have now been obtained using the combinatorial strategy [18]. They are non-peptide analogs. The compound **L054,522** is 3,000 fold more selective for sst2, than for other receptors. It is a potent inhibitor of growth hormone release and of glucagon release *in vivo*.

A betidamino acid scan of Des-AA_{1,2,4,5,12,13} [DTrp₈]-somatostatin-14 led to a family of octapeptide derivatives of somatostatin cyclized via a disulfide bridge with sst3 selectivity [19]. Betidamino acids are N'-monoacylated (optionally, N'-monoacylated and N-mono- or N,N'-dialkylated) aminoglycine derivatives. Each N'-acyl/alkyl group may mimic naturally occurring amino-acid side chains. Structure-activity relationships showed that the basis for sst3 selectivity and high affinity lies in the ring size of the analog and the nature of the N-methylated amino-2-naphthoyl side chain of aminoglycine in position 8. The Tyr₇ substitution or the D-configuration of Trp₃ had no influence on affinity or selectivity. Finally, the acylation of the N terminus with a carbamoyl group yielded the most potent and sst3-selective compound of this series, **sst3-ODN-8**, with an affinity for sst3 equal to that of somatostatin-28 [19]. Sst3-ODN-8 compound was found to be a sst3 receptor subtype antagonist. It was possible to specifically displace sst3-specific binding sites with nanomolar concentrations of compound sst3-ODN-8 in selected human somatostatin

target tissues such as T-cell-rich interfollicular areas of the lymphoreticular system in the tonsils and lymph nodes. There was no specific binding of this compound in the B-cell rich germinal centers of tonsils known to express sst2.

A series of non-peptide derivatives was found using a novel thiourea scaffold to attach a heteroaromatic nucleus to mimic the Trp₈ residue, a nonheteroaromatic nucleus to mimic Phe₇, and a primary amine or other basic group to mimic the Lys₉ residue of somatostatin [20]. In this series, the lead compound, **NNC26-9100**, exhibited a high affinity for sst4 and a sst4 selectivity about 100-fold higher than sst2.

When the backbone cyclization strategy was used in which cyclization is carried out by a lactam bridge between two alkylated residues, **PTR 3046** a peptide compound selective for sst5 was constructed [21]. It inhibited pancreatic exocrine secretion but had no effect on endocrine secretion and growth hormone release from hypothalamus.

3.3 Somatostatin Analogs with a Rather Universal Binding Profile

Instead of developing selective analogs for each sst receptor, the synthesis of universal ligands might be more productive. First, cells generally express several somatostatin receptors activated equally by the natural peptide. Second, several studies demonstrated that G protein-coupled receptors can form heterodimers or hetero-oligomers with specific functional activity, which could be superactivation or inactivation, then exhibiting their own pharmacological characteristics [22]. For example, sst2A and sst3 receptors subtypes exist as homodimers and heterodimers. The sst2A-sst3 heterodimer presents new pharmacological and functional profiles. The first universal analogs described are **KE108** [23] and **SOM 230** [24]. KE108 is a nonapeptide somatostatin analog. It binds to all 5 somatostatin receptors with a very high affinity with agonist properties. The synthesis of cyclohexapeptide somatostatin mimics, and structure activity relationships based on ligand binding studies, led to the discovery of SOM 230. This compound binds to sst1, sst2, sst3, and sst5 receptors with the almost same high affinity and sst4 with a somewhat lower affinity. It inhibits with a high potency the release of GH and that of Insulin-like Growth Factor (IGF1). It is very stable *in vivo* and is currently used in phase I clinical trials.

A number of new peptide or non-peptide compounds with universal binding profile are currently generated [10]. This indicates a great interest for these analogs. However, they are still under evaluation.

3.4 Clinical Use and Side Effects

Analogs available for clinical use are Sandostatin (octreotide), and Somatuline (lanreotide). They are 4 formulations : Sandostatin (Novartis) for subcutaneous or intravenous injection, administrable 3 times a day; Sandostatin LAR (Novartis) that is octreotide incorporated in Poly(D,L-lactide-co-glycolide) microspheres, administrable intramuscularly 1 per 4 weeks; Somatuline LA (Beaufour IPSEN) for intramuscular injections; Somatuline Autogel (Beaufour IPSEN) for intramuscular or subcutaneous injections every 4 weeks.

These analogs are used in hormonal disorders, gastrointestinal disorders and cancer, to treat GH-secreting pituitary adenomas, islet cell tumors and carcinoids. They control symptoms, such as the flushing of metastatic carcinoid tumors and the profuse, watery diarrhea associated with vasoactive intestinal peptide-secreting tumors (VIPomas). The efficacy of somatostatin analogs on pituitary adenomas is long-lasting and does not decrease with time. No long-term desensitization and no rebound at drug withdrawal have been observed. Clinical improvement is obvious in most of patients. However, in some circumstances, the inhibitory effect of somatostatin might decrease rapidly following continuous exposure to somatostatin or analogs [1]. The efficacy of the treatment is variable in patients with TSH secreting adenomas. A loss of sensitivity to the inhibitory effect of octreotide might be observed. Also, desensitization of the effect of somatostatin analogs within weeks or months has been observed in islet cell tumors- or carcinoid-bearing patients. The induction of this tumor type-dependent tachyphylaxis phenomenon might be due to the differential expression of sst receptors, down regulation or, on the contrary, to tissue specific up-regulation of sst receptors other than sst2 [25].

The use of the labelled somatostatin analog octreotide is a widely spread method in clinical medicine as imaging for diagnosis [25]. Neuroendocrine tumors might be detected by scintigraphy using indium labeled octreotide or octreoscan. The presence of sst2 receptors at the membrane level is a prerequisite for the visualization of sst positive tissues using [¹¹¹In-DPTA⁰]-octreotide scintigraphy. Analysis of the uptake of radioactivity reflects internalized radioligand. Indeed, sst receptors and somatostatin internalize following a specific receptor subtype- and agonist-dependent process. These properties have important consequences for visualization of somatostatin receptor positive tumors.

The ability of sst2 receptor to internalize is also used for sst receptor-targeted radiotherapy. Pre-clinical studies indicated that sst receptor-targeted radiotherapy reduced tumor growth in sst-receptor bearing tumors in athymic mice. There is a clinical benefit to the administration of octreotide in patients with tumors and several α -emitting radionuclides, such as ⁹⁰Y, or ¹⁷⁷Lu, are currently tested. Very recently, a new approach of genetic radioisotope targeting has been proposed, based on the induction of sst2 receptor expression and selective tumor uptake of radiolabelled peptides [26].

Some side effects of sst2 analogs are known. They are nausea, vomiting, diarrhea, and malabsorption of fat. These adverse effects are the consequence of the physiological effects of somatostatin. After some weeks of treatment, they disappeared spontaneously. In spite of the inhibition of insulin release, somatostatin did not have any effect on carbohydrate metabolism, probably because of the concomitant inhibition of growth hormone and glucagon release, and the delay in the absorption of carbohydrates. When somatostatin is administered during a long-term treatment, it is associated with the appearance of gallstones. It is due to the inhibition of gallbladder contraction, and the inhibition in cholecystokinetic peptide release (cholecystokinin). Twenty to 30% of patients are concerned.

However, only a very few patients (1%) are symptomatic and need surgery. The effects of other analogs are not known.

Finally, if sst2 analogs are the only compounds to be used in clinical medicine, with an undoubted success, there is a multitude of recently discovered analogs. New analogs binding specifically to receptor subtypes, might lead to the characterization of biological unsuspected activities and new indications of somatostatin. Therefore, the field open by these new analogs should be explored.

4. SOMATOSTATIN AND SOMATOSTATIN RECEPTORS IN INFLAMMATORY DISEASES: CLINICAL, EXPERIMENTAL, AND MOLECULAR ASPECTS

Treatment options using somatostatin analogues in inflammatory chronic diseases have been proposed in a range of chronic inflammatory diseases [27-29]. They have been suggested by clinical facts and experimental data. Molecular mechanisms to explain the modes of actions of somatostatin have begun to be elucidated.

4.1 Expression of Somatostatin and Somatostatin Receptors in Inflamed Organs

Rheumatoid arthritis is a chronic inflammatory disease that primarily affects small joints of hands and feet [30]. It is characterized by inflammation of the synovium and destruction of local articular structures. It has been suggested to be an autoimmune disease. Various therapeutic strategies have been designed, on the basis of various pathogenic mechanisms. However it is still a major cause of disability. Somatostatin and somatostatin analogues have been shown to be effective in the treatment of patients with rheumatoid arthritis [31]. Somatostatin reduced progressively joint inflammation. Intra-articular injections of somatostatin-14 reduced significantly synovial thickness in rheumatoid arthritis [32], as assessed by reduction of pain. Another, although indirect, argument concerns the case of the onset of rheumatoid arthritis following curative surgical treatment of somatostatinoma in a patient [33]. The normalization of plasma somatostatin levels after surgery was followed by the apparition of rheumatoid arthritis, indicating that somatostatin could exert a protective effect.

Recently in 2001, a pilot study of a long acting somatostatin analogue, sandostatin, evaluated its efficacy and safety, for the treatment of refractory rheumatoid arthritis [34]. Ten patients were included. The treatment led to a significant clinical improvement leading to the classification of sandostatin as a potential "Disease Modifying Antirheumatic Drug".

There are several animal models for experimental arthritis, reproducing the human disease. In rabbits, somatostatin treatment induced a statistically significant and dose related reduction of knee joint swelling [35]. The effect of somatostatin was comparable to that of triamcinolone. Triamcinolone appeared to be more effective but its effect declined in extent and duration while the effect of somatostatin remained unchanged at each successive treatment. In rats, octreotide shows an anti-inflammatory

effect in experimental arthritis induced by Freund's adjuvant, but it is weaker in comparison to dexamethasone [36]. The addition of octreotide to dexamethasone does not result in a more pronounced anti-inflammatory action of glucocorticoid alone.

The action of somatostatin in arthritis rheumatoid is explained by paracrine or autocrine regulation of synovial cells functions [31].

During rheumatoid arthritis, the synovium, which is normally a relatively acellular structure is invaded by CD4⁺T cells, B cells and macrophages. sst receptors-expressing cells have been identified using sst receptor antibodies. sst2 receptors were expressed by the endothelial cells of the synovial veinules and also by a subset of synovial macrophages [29]. sst1 and sst2 receptors were expressed on fibroblast synovial cells and the expression of sst2 receptors was up-regulated by proinflammatory cytokine treatment of the synovial cells in arthritic patients [37].

Synovial cells produced locally pro-inflammatory cytokines and degradative enzymes such as matrix metalloproteinases (MMP_s) [30]. Somatostatin itself is produced locally in ankles of rats with adjuvant arthritis or in rheumatoid arthritis patients. Increased levels of somatostatin have been found by radioimmunoassay [38] and somatostatin producing cells have been identified using immunohistochemical labeling and electron microscopy. In rheumatoid arthritis patients, increased somatostatin content was found in the mature bone matrix, monocytes and polymorphonuclear cells of bone marrow, and macrophage-like synovial cells. Fibroblast-like cells synthesized somatostatin by themselves [37].

Thus, somatostatin could activate sst receptors to inhibit proliferation of synovial cells, production of proinflammatory cytokines and MMP_s.

Sarcoidosis and Granulomas

Focal chronic granulomatous inflammation is the consequence of a normal immune response to persistent noxious substances and antigens released by helminthic ova. In murine *Schistosomiasis mansoni*, helminthic worms live in the portal and mesenteric veins. They produce ova that are deposited in the liver and intestinal walls. In humans, the infection is responsible for severe hepato-splenic fibrosis. There is no complete treatment for schistosomiasis. Since somatostatin is produced by granulomas and endogenous levels of plasma somatostatin are inversely correlated with morbidity in patients with *Schistosoma mansoni*, it has recently been concluded to the efficacious therapeutic potential of somatostatin [39].

Experimental data confirmed the effects of somatostatin on the hepatic consequences of the infection. It is well known that octreotide diminishes the size of murine hepatic granulomas elicited by eggs of the parasite *Schistosoma mansoni*, and reduces portal pressure and weight of the spleen. Somatostatin could inhibit in some conditions the proliferation of hepatic stellate cells and transdifferentiation in myofibroblastic phenotype [40].

Granuloma is a unique model to delineate cytokine circuitry at the site of chronic inflammation [41]. It is

composed of T cells, B cells macrophages, eosinophils and other cell types. The granulomas represent strong Th2 (type 2 helper T cell) responses producing large amounts of IL-4 and IL-5. They also produce Th1 cytokines, IL-12 and some Interferon (IFN). Somatostatin receptors have been detected in the inflammatory lesions of patients suffering from sarcoidosis and other granulomatous diseases. Sst2 is the only receptor described in granuloma cells. It is expressed by T-lymphocytes, macrophages, epithelioid cells and giant cells [41]. No PCR-amplified product from granuloma cells for sst1, sst3, sst4, and sst5 mRNA has been found [42].

It has been clearly demonstrated by several groups that somatostatin is temporarily produced by granulomas in mice cysticercosis, a parasitic infection. Macrophages are somatostatin producing cells [43]. They express the preprosomatostatin mRNA and contain the 14 amino-acid form of somatostatin as detected by radio-immunoassay or immuno-histochemistry. Somatostatin produced by macrophages is acting locally on somatostatin receptors [44], to inhibit IFN and IgG2 release by antigen-stimulated granuloma cells and splenocytes of schistosoma-infected mice [42]. This effect is directly dependent on sst2 receptor activation, since sst2 specific antiserum blocks the somatostatin and octreotide effect on IFN and IgG2 release.

Therefore, in mice, the immuno-regulatory circuit at the site of inflammation is composed of 1) macrophages of the granulomas producing large amounts of somatostatin [41], after stimulation by IFN and Tumor Necrosis Factor (TNF) or other mediators of inflammation and 2) lymphocytes expressing sst2 receptors. Somatostatin acts on sst2 receptor to down-regulate IFN production.

Graves Ophthalmopathy

Graves disease is a thyroid specific autoimmune disease. Recent progress have been made for a comprehensive pathogenesis of the disease which is probably multifactorial. Therapeutic strategies by immunosuppressive agents especially glucocorticoids are far from being satisfactory. Therefore, the long acting somatostatin analog has been proposed to treat the ophthalmopathy associated with Graves disease. It was effective in reducing soft tissue inflammation, improving extra-ocular muscle function and providing symptomatic treatment. Compared to corticosteroids, somatostatin had similar efficacy and minimal side-effects [45].

In Graves disease, somatostatin receptors have been identified in lymphocytes from retro-orbital tissues. The expression of sst1-5 genes was evaluated by PCR amplification on lymphocytes from blood samples aspirated in orbits or recovered from retro-orbital tissues during surgery [46]. Lymphocytes from retro-orbital tissues or blood samples express all five sst receptors. Major expression was found with sst1, then equally sst2 and sst4, sst3 and sst5 receptors being expressed at a lower level. Lymphocytes from control samples showed no or very low expression of sst_s. Retro-orbital fibroblasts express somatostatin along with sst_s [47] and octreotide inhibited their proliferation.

Inflammation in the Digestive Tract

Several elements indicate that somatostatin could potentially have benefic effects on intestinal inflammatory diseases. It has been demonstrated that the secretion of proinflammatory cytokines by intestinal cells could be regulated by somatostatin. In experimental chronic colitis caused by trinitrobenzene sulphonic acid in rat, a model of Th1 disease, somatostatin inhibits TNF expression and inducible Nitric Oxide Synthase (NOS) from submucosal macrophages, and colonic production of IL-1 and IFN [48]. The secretion of IL-8 and IL-1, caused by TNF or bacteria in intestinal cell lines is abrogated by octreotide [49]. Also, the decreased content of immuno-reactive somatostatin in rectal mucosa of patients with inflammatory bowel disease has been suspected to be involved in the onset of the disease [50].

In inflammatory bowel diseases, Crohn Disease or Ulcerative Colitis in humans, sst receptors are expressed in epithelial cells, peripheral nervous system, gut associated lymphoid tissue, and in most intra-mural intestinal veins, but not in arteries [51]. They are undetectable in the veins of non-inflamed tissues [51,52]. However, the role of somatostatin in intestinal inflammation could not be confirmed. The effects observed in inflammatory bowel disease or pancreatitis are largely controversial. In patients with severe ulcerative colitis, octreotide is not of additional benefit as adjuvant therapy to high doses of corticosteroids [53]. In patients with acute pancreatitis, the effect of somatostatin and stable analogs remains an open question [54]. Neither acute nor chronic pancreatitis seem to benefit of somatostatin treatment [55,56]. However, a recent study shows that lanreotide prevented pain relapse after oral refeeding in patients with acute pancreatitis [57].

Other Local Experimental Inflammation

Octreotide and lanreotide were found to be active in suppressing inflammation caused by carrageenin-induced inflammatory responses in rats [58], by reducing the volume of exudates. Octreotide possesses antiphlogistic activity in zymosan-induced mice ear inflammation, roughly comparable with classical anti-inflammatory drugs such as dexamethasone or ketoprofen [59]. In this last model, cyclic synthetic somatostatin analogs such as TT-232, were proved to be more effective than octreotide in inhibiting inflammatory processes [13].

4.2 Expression of Somatostatin and Somatostatin Receptors in the Immune Cell System

In addition to the local expression of somatostatin and sst receptors in inflamed organs, cells of the immune system - thymus, spleen, and peripheral blood immune cells - express somatostatin and sst receptors as well, and somatostatin or analogs can regulate their functions.

Thymus

The thymus is a central organ in lymphoid system. It involutes during the first ten years of life but part of its activity is still maintained throughout life. It is composed of 4 types of cells : thymocytes, macrophages, dendritic cells and thymic epithelial cells. Thymocytes are the lymphocytes

of the thymus. They differentiate into mature peripheral T-lymphocytes by the interaction with the three other cell types present in the thymus.

Experiments using labelled somatostatin or gene amplification by RT-PCR showed that sst1, sst2 and sst3 could be characterized in the whole human thymocyte population [60-62]. After separation into subpopulations, it was found that sst2 and sst3 are differentially expressed in intermediate/mature and immature thymocytes. The expression of sst2 mRNA was higher than that of sst3 in the immature CD2+CD3- fraction [63], whereas the expression of sst3 mRNA was higher than that of sst2 in the intermediate/mature CD3+ fraction. Thymic epithelial cells expressed sst2 mRNA and in a less important amount sst1 [64,65]. Neither sst4 nor sst5 were found in thymocytes [61] or thymic epithelial cells [65]. The monocyte-macrophage lineage, composed of CD14+ thymic cells, selectively expressed sst2 mRNA.

Somatostatin itself could be produced in normal thymus. Somatostatin mRNA is detected in normal murine thymic epithelial cells [66], in human thymic tissue and human cultured epithelial cells [60,65], indicating that produced somatostatin could interact directly with any sst receptors, by an autocrine loop. One of the most interesting finding of these last years, is the discovery of the presence of cortistatin in human immune cells [7]. Cortistatin, at a higher rate than somatostatin, is produced preferentially by thymic epithelial cells in human.

Accordingly, somatostatin significantly inhibited [³H]thymidine incorporation in thymic epithelial cells [63]. sst3 but not sst2, might be involved in the mature thymocyte apoptotic process and plays an important role in thymocyte functions [66]. Moreover, sst3 has recently been characterized on peripheral human T lymphocytes, which derive directly from mature thymocytes [64], and somatostatin analogs may induce apoptosis in these cells. A recent study [66] demonstrated that somatostatin triggers chemotactic responses on thymocytes, and enhances thymocyte adhesion to a stromal cell line.

In summary, the effects of somatostatin which could be autocrine/paracrine effects are potentially 1- to control thymic epithelial cell proliferation, 2- to regulate the migration of immune cells in different compartments favoring the traffic of immature cells toward peripheral lymphoid organs, and 3- to control the thymic involution process.

Spleen

Normal rat spleen contains sst1, sst3 and sst4, whereas normal mice spleen contains sst1 and sst4 [67]. Splenocytes from *schistosoma* infected animals do contain sst3 or sst4 mRNA [67], as well as sst2 receptors. The activation of sst2 inhibited the release of IFN and IgG2 by T-cells in these infected animals. In human, the spleen shows the highest uptake of labelled octreotide.

Although splenocytes from normal mice did not express preprosomatostatin mRNA, the messenger is inducible in inflammatory conditions. In splenocytes from normal mice, somatostatin mRNA is induced by LPS, IFN, or IL-10, and

the protein was identified by immunochemistry [68]. This phenomenon is also found *in vivo*: the preprosomatostatin mRNA is induced in splenocytes from normal mice by cytokines and inflammatory mediators (IL-10, IFN γ , TNF α , prostaglandin E₂, VIP, and dibutyl cyclic AMP) or in splenocytes from *Schistosomiasis* animals [44]. This induction is observed even in SCID mice lacking B and T-cells. Somatostatin producing cells in mice spleen are probably of macrophage origin [68].

Peripheral Blood Immune Cells

In physiological state, somatostatin receptors are expressed on peripheral blood immune cells. T-cells, B-cells and monocytes [69] are sst₅-expressing cells. Granulocytes are the only cell type that do not express sst receptors.

Two important variations have been demonstrated in the expression of receptor subtypes in peripheral blood immune cells: they are largely depending on cell activation and species [62,67].

In resting normal human peripheral blood, somatostatin receptors are found on T- and B-lymphocytes and monocytes [69]. They are of the sst₃ subtype and of moderate affinity for octreotide. When lymphocytes or monocytes are mitogen-activated, somatostatin receptors are of the sst₂, sst₃ or sst₅ subtype and display low or high affinity for octreotide [28,67,70]. By contrast, in the rat, during experimental arthritis, cells of the immune system appear to express a different profile of sst₅, which are composed of sst₃ and sst₄ [71]. There is an higher expression of sst₄ receptors in rat monocytes. No binding of sst₂ and sst₅ selective radioligand was found in accordance with the lack of sst₂ and sst₅ expression as detected by RT-PCR in monocytes [71].

The expression of sst receptors on precursor cells is not well studied. In human bone marrow, sst₂ is exclusively expressed, mostly on a small subset of cells, such as the CD34⁺ fraction [72]. This fraction includes the pluripotent stem cells and early progenitors. Interestingly, somatostatin mRNA is not detected in human macrophages and monocytes, whereas a strong expression of corticostatin was found [73]. The activation of monocytes upregulated the expression of cortistatin. It has been recently demonstrated that a selective induction of cortistatin occurred in activated human monocytes, leading to the idea that cortistatin could play a most important role, rather than somatostatin, in the human immune system.

Thus, the effects of somatostatin are: 1- to regulate monocyte and macrophages functions. The immunosuppressor effects of somatostatin were examined on purified peripheral blood human monocytes [74]. Somatostatin, at concentrations thought to be physiologic (0.1-100 nM), regulated monocyte/macrophage responses to LPS stimulation, as reflected by interleukin production. It has direct inhibitory effects on TNF α , IL-1 β , and IL-6 secretion by LPS-activated monocytes. It reduced the production of IFN γ from human peripheral mononuclear cells [28] and suppressed superoxyde release from human monocytes. 2- to inhibit T lymphocyte functions, *in vivo* and *in vitro*. Somatostatin interacts directly with its receptors on T-cells to inhibit the release of proinflammatory cytokines

[75,76]. It drives T-cells into the secretion of cytokines, transforming the Th phenotype: secretion of Th1 cytokines from a Th2 cell line and vice versa [77]. The production of IFN γ , a pro-inflammatory cytokine belonging to the Th1 cytokine group, that enhances the cellular immune response to intracytoplasmic pathogens, is decreased and that of IL-2 is increased. Also, somatostatin inhibits lymphocyte proliferation *in vitro* [78], especially when purified T-lymphocytes or T-cell lines are used [28]. Somatostatin inhibited cell proliferation in the Jurkat line of human leukemia T-cells. Octreotide enhanced the immunosuppressive effect of FK506 [76], leading to an immunosuppressive effect without undesirable side effects. Somatostatin might synergize with immunosuppressors by interfering with different pathways of T-cell activation. 3- to inhibit B-lymphocyte function. *In vivo* treatment by somatostatin in rats with chronic inflammatory disease have shown that the production of immunoglobulins, especially Ig2a, is decreased by somatostatin [28]. Somatostatin is thought to have an effect on B-cell activation, proliferation or differentiation and inhibits the synthesis of IgA but not IgM or IgG in antibody producing cells. Octreotide is active indicating that sst₂ and/or sst₃ and/or sst₅ are involved.

4.3 Scintigraphy in Inflammatory Diseases

Following the proven clinical importance of octreoscan to visualize sst receptor positive tumors, it has been proposed to use it to evaluate the extent of immune-mediated diseases and their responses to therapy [79].

Somatostatin receptors has been visualized in human rheumatoid arthritis inflamed joint, or in experimental arthritis, by *in vivo* scintigraphy using the sst₂ and sst₅ selective somatostatin analog [¹¹¹In-DTPA-D-Phe1]-octreotide [80]. None of the joints from control patients demonstrated uptake of radioactivity. The presence of somatostatin receptors was confirmed by the binding of iodinated octreotide to synovial membranes from patient with rheumatoid arthritis, indicating that cells of the inflammatory infiltrate in the joint possess sst subtype receptors which have a high affinity for octreotide, sst₂, sst₅, or sst₃ [80].

The tissue distribution of indium-labelled octreotide binding to receptors in a murine model of immune mediated disease, was confirmed by *in vitro* autoradiography, indicating that mouse could be an animal model for preclinical evaluation of the effects of somatostatin analogs in the immune system [81], at least in some pathological conditions. Very recently, sst scintigraphy was found to be useful to manage ANCA-associated vasculitis, being an indicator of activity and extent of the disease and treatment efficacy [82].

It is in patients with Graves disease that octreoscan has been shown to be most important in clinical medicine. In thyroid-associated orbitopathy, orbital scintigraphy is a method of choice to establish or to follow the therapeutic strategy. Scintigraphic imaging with indium-111 pentreotide [83] is used to evaluate the clinical activity of the orbital disease. The binding of octreotide is well correlated with ophthalmopathy permitting to select patients for immunosuppressive treatment and /or octreotide.

Accumulation of radio-labelled somatostatin analog in retro-orbital tissue has been demonstrated *in vivo* by octreotide scintigraphy suggesting the presence of specific binding sites for octreotide on the orbit components, fibroblasts and/or infiltrating immune cells.

5. MECHANISMS OF ACTIONS OF SOMATOSTATIN. INTRA-CELLULAR PATHWAYS AND RELEVANCE TO FUNCTIONS

Using recombinant sst_s expressed in various eukaryotic cells and specific receptor subtypes analogs, further progress have been made in understanding the intracellular signal transduction machinery triggered by somatostatin. The binding of somatostatin to sst_s caused a wide variety of pertussis-toxin sensitive and insensitive G-protein dependent intra-cellular signals, each receptor subtype being coupled to multiple intracellular transduction pathways.

The anti-inflammatory properties of somatostatin and analogs might be explained by antisecretory effects, antiproliferative effects on immune or non-immune cells, antiangiogenic properties, and regulation of cell migration.

5.1 Antisecretory Activity

The inhibition of neurotransmitter and hormone release by somatostatin is depending on inhibition of adenylate cyclase and/or regulation of ion channels. This has been reviewed extensively [84]. All five sst_s are functionally coupled to the inhibition of adenylate cyclase via a pertussis toxin sensitive protein. The Gi₁₋₃ are the G-proteins involved in the inhibition of cyclic AMP formation. Furthermore, these receptors are functionally coupled to different voltage dependent channels. Sst1, sst2 and sst5 decrease Ca²⁺ influx by directly inhibiting high voltage-dependent Ca²⁺ channels via Go_{2/1/3}. Somatostatin regulates different voltage-dependent Ca²⁺ currents, including L-type, N-type and T-type currents. Thus somatostatin-induced inhibition of peptide secretion mainly results from a decrease in intracellular Ca²⁺. This is also achieved by opening K⁺ channels, via Gi₂ or Gi₃ [84]. Somatostatin regulates several voltage-gated K⁺ currents. sst2, sst3, sst4, and sst5 activate Inward Rectifying Potassium channels whereas sst1 seems to be involved in the inhibition of K⁺ currents.

5.2 Inhibition of Cell Proliferation

This property of somatostatin has been characterized more recently but it is now largely recognized. It could be secondary to cell growth arrest and/or apoptosis.

5.2.1 Cell growth Arrest

Several mechanisms have been proposed to explain cell growth arrest caused by somatostatin : inhibition of Na⁺-H⁺ exchanger type 1 (NHE1), stimulation of PTP_s and regulation of Mitogen-Activated Protein Kinase (MAPK).

- Somatostatin inhibits the activity of NHE1. NHE1 is ubiquitously expressed. It allows Na⁺ to enter the cells in exchange for H⁺, leading to an alkalization of intra-cellular pH, that is associated with cell proliferation. When heterogeneously expressed in fibroblasts, sst1, sst3 and sst4

inhibit NHE1 by a GTP-dependent but Gi-independent mechanism. Recently, T/S/P-V consensus motifs within the intra-cellular domain 2 and Q-Q/R within the intra-cellular domain 3 of sst1, sst3, or sst4 have been demonstrated to directly couple GPCR_s to NHE1. Interestingly these motifs are absent in sst2 and 5 which do not signal to NHE1.

- Somatostatin and analogs activate a number of PTP_s including the SH2 domains-containing non-transmembrane PTP_s, SHP-1 and SHP-2, serine/threonine phosphatases, the Ca²⁺-dependent phosphatase, calcineurin, and the receptor-like PTP, r-PTP_C. Antiproliferative actions of somatostatin have been shown to result partly from translocation and/or activation of SHP-1 in various cell systems expressing endogenous sst2 [84]. SHP-1 associates *in vivo* with activated growth factors tyrosine kinase receptors, or activated cytokine receptors, or receptor complexes of the immune system, to dephosphorylate critical signal transduction molecules for these receptors [85]. SHP-2 is directly associated with phosphorylated Tyr₂₂₈ and Tyr₃₁₂ located in sst2 ITIM_s. Upon sst2 activation by somatostatin, SHP-2 is activated and permits in turn SHP-1 recruitment and activation. Then, activated SHP-1 rapidly dissociates from sst2 to be recruited by its substrates leading to their dephosphorylation, negative regulation of mitogenic signaling, and inhibition of cell proliferation [4].

- All five sst_s are coupled to MAPK pathways. Sst2 inhibits Extracellular signal-Regulated Kinase (ERK) 1/2 activity and cell proliferation induced by growth factors in a PTP-dependent manner [84]. Likewise, sst3 inhibits the ERK1/2 pathway via PTP-dependent inactivation of Raf-1 in transfected NIH3T3 cells. Sst5-mediated inhibition of ERK1/2 results from an inhibition of guanylyl cyclase and cGMP-dependent protein kinase [86]. However, somatostatin, acting through sst2, could also activate ERK1/2 in association with an antiproliferative effect. When transfected in CHO-DG44 cells, sst2 inhibits the mitogenic insulin effects through a strong and transient stimulation of ERK1/2 activity, which is dependent on a Ras-Rap1/B-Raf/MEK pathway. Furthermore, SHP-1 and SHP-2 are required upstream of Ras and Rap1. Also, sst1-dependent ERK1/2 activation leads to the inhibition of cell proliferation through stimulation of SHP-2, Ras and Raf-1 activities. These pathways are involved in the expression of the cyclin-dependent protein kinase inhibitors p21^{Cip1} or p27^{Kip1} resulting in the inhibition of cell proliferation [87]. Finally, sst4 induces a sustained activation of ERK1/2 leading to Ras-independent cell proliferation [84]. Thus, the MAPK ERK pathway is an important mediator of somatostatin-induced cell growth regulation, leading to the inhibition of cell proliferation, except for sst4.

5.2.2 Induction of Apoptosis

In some circumstances, the inhibition of cell proliferation caused by somatostatin results from the induction of an apoptotic cell death program. Somatostatin can promote apoptosis in normal and cancer cells. Until now, only sst2 and sst3 have been involved in this effect via two main signaling pathways, so-called cell-extrinsic pathway and cell-intrinsic pathway.

The cell-extrinsic pathway sensitizes cells to apoptosis induced by TNF family death receptors. The cell-intrinsic pathway triggers apoptosis through activation of the pro-apoptotic Bcl-2 gene superfamily, in response to DNA damage, or loss of cell survival factors. The activation of caspases is involved in both pathways. Strikingly, sst2 affected both death ligand and mitochondria pathways, by up-regulating the expression of the TNF-related Apoptosis Inducing Ligand (TRAIL) and TNF receptors, DR4 and TNFR1 (TNF receptor 1) and down-regulating the expression of the antiapoptotic mitochondrial Bcl-2 protein. SHP-1 has been shown to be a critical component for somatostatin induced apoptosis [88] and acidification in breast carcinoma cells [89]. In CHO-K1 cells, sst3 is the only receptor that induces apoptosis, in association with dephosphorylation-dependent conformational changes of p53 and induction of Bax [90]. The apoptotic program is triggered by somatostatin in activated peripheral blood lymphocytes [78]. The cleavage of poly-ADP ribose polymerase (PARP), the activation of caspase-3-like activity and the fragmentation of DNA were observed in peripheral blood lymphocytes stimulated by low doses of octreotide.

5.2.3 Inhibition of Angiogenesis

Angiogenesis is the process by which new blood vessels are formed. It plays an important role in inflammatory diseases. For example, rheumatoid arthritis is characterized by excessive angiogenesis. Clearly, the inhibition of angiogenesis may lead to the inhibition of inflammatory cell proliferation and thus inflammatory lesions [29]. Inhibitors of angiogenesis have been shown to be effective in preventing neovascularization of the joint.

Somatostatin has been shown to be a potent inhibitor of angiogenesis *in vitro* and *in vivo* in a number of experimental models. It inhibited the proliferation of endothelial cells, human umbilical vein endothelial cells (HUVEC) [91] and murine endothelial cells (HECa10) [92]. Neovascularization is inhibited by somatostatin in the chicken chorioallantoic membrane (CAM) model and in the human placental vein model (HPVAM) [91]. This inhibition appears to be the result of a unique up-regulation of sst2 during the angiogenic switch from resting to proliferating endothelium [91]. In other cell systems, such as Bovine Artery Endothelial Cells (BAEC), expressing sst1, 3, and 5, or human endothelial cells, EAhy926, expressing only sst3, the anti-angiogenic activity of somatostatin appeared to be mediated by interaction with sst3 [93].

The question as to whether the anti-angiogenic effect of somatostatin is exerted through the inhibition of growth factor secretion such as IGF-I and vascular endothelial growth factor (VEGF) and/or inhibition of growth factor-induced VEGF synthesis has been explored. Results indicated that *in vitro* somatostatin suppressed VEGF synthesis, but did not modified VEGF secretion [92].

Endothelial cells require MAPK activation and vasodilating properties of Nitric Oxide (NO) to proliferate. In addition to the inhibition of MAPK activity, an inhibition of endothelial NOS activity is required for somatostatin to cause an inhibition of angiogenesis [93]. The control of NO-mediated vasodilatation is an important target of

somatostatin. In inflammatory bowel diseases [94], NO is no more able to cause vasodilatation in microvessels, due to an acquired microvascular dysfunction (Crohn disease and ulcerative colitis). In these diseases, there is a loss of response to somatostatin [94].

5.3 Regulation of Cell Migration

The effects of somatostatin on homing and migration of human monocytes are unique. They have recently been demonstrated. Octreotide seems to act as a chemotactic and chemokinetic agent [73]. Chemoattraction is the attraction exerted by a compound towards cells which move in the direction of a positive gradient, whereas chemokinesis reflects activation of cell motility and migration at random. The activation of sst2 by octreotide may act as a potent promigratory stimulus for CD34+ bone marrow cells *in vitro* and has an effect on the migration of normal cells *in vivo* favoring the migration of normal hematopoietic progenitors in the inflamed tissues. In normal peripheral T-cells, the activation of sst3 or sst2 favors T-cell adhesion to fibronectin, a major glycoprotein component of extra-cellular matrix [82], and, at a lesser extent, adhesion to collagen type IV and laminin [95]. The somatostatin-induced T-cell adhesion to fibronectin is mediated by the $\alpha 4 \beta 1$ and $\alpha 5 \beta 1$ integrins. The expression of integrins is not modified but conformational changes are probably induced by the activation of sst. The interaction of T-cells with blood vessel walls leads subsequently to their migration to sites of inflammation. By contrast, in fibroblasts, it has recently been demonstrated that sst1, but not sst2, inhibits Rho activity, the assembly of focal adhesion and actin stress fibers and cell migration [96]. The inhibition of Rho activity is in agreement with the downstream inhibition of NHE1, that is phosphorylated by the Rho-associated kinase. Consequently, the activation of sst1, results in an inhibition of thrombin stimulated endothelial cell migration, suggesting that sst1 might be involved in the antiangiogenic activity of somatostatin.

6. CONCLUSIONS

Somatostatin and octreotide have been classified as potential anti-inflammatory agents [27-29]. The rationale for somatostatin treatment in chronic inflammatory diseases is clear. Somatostatin receptors have been identified on lymphocytes (activated peripheral blood lymphocytes, lymphocytes from immune organs, and lymphocytes at the local site of inflammation, such as joints, granulomas, orbits) and other cells participating in the inflammatory responses. Anti-secretory, anti-proliferative and anti-angiogenic properties of somatostatin are crucial to control the inflammatory process. Somatostatin reduces synovial cell proliferation in rheumatoid arthritis patients and retro-orbital fibroblast proliferation in Graves disease. It controls angiogenesis, an event playing a major role in the development of inflammation. In addition, the regulation of cytokine release, which could be exerted directly or indirectly by somatostatin, contributes to treat the immune related features of inflammation.

However, the final proof of the activity of somatostatin analogs in clinic is still missing, and reports might be

controversial. This might be due to the lack of available pharmacological tools and to the great diversity of somatostatin triggered pathways. If recent advances in somatostatin chemistry led to an impressive number of analogs, there are a very few somatostatin analogs (octreotide, lanreotide and vapreotide) available for clinical use, showing all combined high affinity for sst2/sst5/sst3 receptors. New compounds specific for a given subtype or universal analogs should be evaluated for their potential to treat inflammatory diseases. The diversity of somatostatin regulatory pathways is due to the fact that cells generally express several receptor subtypes, and several routes are usually involved in the activity of a single receptor, all receptors being activated by the natural peptides. This great complexity is leading to a biological selectivity, probably resulting from the cell type and the differential expression of receptor subtypes, and which is far from being explored. A sst receptor selectivity for antiangiogenic and antiproliferative properties has been observed in experimental models, but selectivity for cytokine release has never been explored. It could be similar to the selectivity showed by somatostatin to inhibit hormone release. So far, it is impossible to predict which analog might be most important. Sst2 is no more the only sst to be targeted. The role of other receptors appears to be of increasing importance.

Another interesting aspect of somatostatin is the existence of an autocrine or paracrine ligand-receptor regulatory pathway in chronic inflammatory diseases. Indeed, the expression of somatostatin receptors is often associated with the production of somatostatin itself by the same cells or neighboring cells. It is a common mechanism of signal transduction that is crucial to control cellular response leading to the permanent production of endogenous, active peptide, at concentrations sufficient to activate receptors. It has been shown to have a great importance in cancers, when dysregulation of this autocrine pathway generates uncontrolled proliferation. A somatostatin negative autocrine loop has already been described and demonstrated to play a major role in pancreatic cancer [97]. Therefore, it is assumed that this pathway might be important in chronic inflammatory diseases. Its role should be investigated.

Also, the physiological and/or patho-physiological role of cortistatin, suspected to be an endogenous ligand for somatostatin receptors in the human immune system, should be elucidated. An autocrine corticostatin-sst2 regulatory pathway has been described in immune human system, instead of the somatostatin-sst2 regulatory pathway identified in immune murine system, leading to the assumption that cortistatin might be a more important regulator than somatostatin in the immune system. Therefore, the search for analogs, based on corticostatin would be rewarding.

Some chronic inflammatory diseases, such as rheumatoid arthritis, are still a major clinical problem. More efficacy and less secondary effects in therapeutic schemas are required. Therefore, the discovery of further therapeutic approaches to control these diseases is needed. Somatostatin analogs are candidates to play a role in this schema. The interest of somatostatin would be minor secondary effects, no risk of

infection as shown by some cytokines, and possibly long-term use like in acromegaly. Also, somatostatin might synergize with other drugs leading to a significant improvement in the resulting therapy with less doses and adverse effects. Synergy might also occur between the different sst_s to exert more potent signals.

Finally, anti-proliferative and anti-angiogenic properties of somatostatin, involved in the control of chronic inflammatory diseases, are also important to control the tumoral process [98]. A role of somatostatin in the treatment of tumor cell growth of neoplastic or non-neoplastic origin is currently clinically explored. Therefore, research on analogs with anti-inflammatory properties might increase the spectrum of available therapy for other diseases including cancer.

LIST OF ABBREVIATIONS

BAEC	=	Bovine Artery Endothelial Cells
CAM	=	Chorio-Allantoic Membrane
CRH	=	Corticotropin-Releasing Hormone
ERK	=	Extracellular Regulated Kinase
GH	=	Growth Hormone
GPCR	=	G-Protein Coupled Receptor
HPVAM	=	Human Placental Vein Angiogenesis Model
HUVEC	=	Human Umbilical Vein Endothelial Cell
IFN	=	Interferon
ITIM	=	Immunoreceptor Tyrosine-based Inhibitory Motif
IGF1	=	Insulin-like Growth Factor 1
LPS	=	Lipo-PolySaccharide
MAPK	=	Mitogene-Activated Protein Kinase
MMP	=	Matrix Metallo-Proteinase
NHE	=	Na ⁺ -H ⁺ Exchanger
NO	=	Nitric Oxide
NOS	=	Nitric Oxide Synthase
PTP	=	Phospho-Tyrosine Phosphatase
SRIF	=	Somatotropin-Releasing Inhibitory Factor
sst _s	=	All somatostatin receptor subtypes
sst _x	=	Somatostatin receptor subtype where x is the subtype number
TNF	=	Tumor Necrosis Factor
TNFR1	=	TNF Receptor 1
TRAIL	=	TNF-Related Apoptosis Inducing Ligand
TRH	=	Thyrotropin-Releasing Hormone
TSH	=	Thyroid-Stimulating Hormone
VEGF	=	Vascular Endothelial Growth Factor
VIP	=	Vasoactive Intestinal Peptide

VIPomas = Vasoactive Intestinal Peptide-secreting tumors

REFERENCES

- [1] Patel, Y.C. *Front Neuroendocrinol*, **1999**, *20*, 157.
- [2] Pradayrol, L.; Jornvall, H.; Mutt, V.; Ribet, A. *FEBS Lett.*, **1980**, *109*, 55.
- [3] Lin, C.Y.; Varma, M.G.; Joubel, A.; Madabushi, S.; Lichtarge, O.; Barber, D.L. *J. Biol. Chem.*, **2003**, *278*, 15128.
- [4] Ferjoux, G.; Lopez, F.; Esteve, J.P.; Ferrand, A.; Vivier, E.; Vely, F.; Saint-Laurent, N.; Pradayrol, L.; Buscail, L.; Susini, C. *Mol. Biol. Cell*, **2003**, *14*, 3911.
- [5] de Lecea, L.; Criado, J.R.; Prospero-Garcia, O.; Gautvik, K.M.; Schweitzer, P.; Danielson, P.E.; Dunlop, C.L.; Siggins, G.R.; Henriksen, S.J.; Sutcliffe, J.G. *Nature*, **1996**, *381*, 242.
- [6] Fukusumi, S.; Kitada, C.; Takekawa, S.; Kizawa, H.; Sakamoto, J.; Miyamoto, M.; Hinuma, S.; Kitano, K.; Fujino, M. *BioChem. Biophys. Res. Commun.*, **1997**, *232*, 157.
- [7] Dalm, V.A.; van Hagen, P.M.; van Koetsveld, P.M.; Langerak, A.W.; van der Lely, A.J.; Lamberts, S.W.; Hofland, L.J. *J. Clin. Endocrinol. Metab.*, **2003**, *88*, 270.
- [8] Robas, N.; Mead, E.; Fidock, M. *J. Biol. Chem.*, **2003**, *278*, 44400.
- [9] Janecka, A.; Zubrzycka, M.; Janecki, T. *J. Pept. Res.*, **2001**, *58*, 91.
- [10] Weckbecker, G.; Lewis, I.; Albert, R.; Schmid, H.A.; Hoyer, D.; Bruns, C. *Nat. Rev. Drug Discov.*, **2003**, *2*, 999.
- [11] Liebow, C.; Reilly, C.; Serrano, M.; Schally, A.V. *Proc. Natl. Acad. Sci. USA*, **1989**, *86*, 2003.
- [12] Keri, G.; Mezo, I.; Horvath, A.; Vadasz, Z.; Balogh, A.; Idei, M.; Vantus, T.; Teplan, I.; Mak, M.; Horvath, J.; et al. *BioChem. Biophys. Res. Commun.*, **1993**, *191*, 681.
- [13] Helyes, Z.; Pinter, E.; Nemeth, J.; Keri, G.; Than, M.; Oroszi, G.; Horvath, A.; Szolcsanyi, J. *Br. J. Pharmacol.*, **2001**, *134*, 1571.
- [14] Afargan, M.; Janson, E.T.; Geleman, G.; Rosenfeld, R.; Ziv, O.; Karpov, O.; Wolf, A.; Bracha, M.; Shohat, D.; Liapakis, G.; Gilon, C.; Hoffman, A.; Stephensky, D.; Oberg, K. *Endocrinology*, **2001**, *142*, 477.
- [15] Gazal, S.; Geleman, G.; Ziv, O.; Karpov, O.; Litman, P.; Bracha, M.; Afargan, M.; Gilon, C. *J. Med. Chem.*, **2002**, *45*, 1665.
- [16] Rohrer, S.P.; Birzin, E.T.; Mosley, R.T.; Berk, S.C.; Hutchins, S.M.; Shen, D.M.; Xiong, Y.; Hayes, E.C.; Parmar, R.M.; Foor, F.; Mitra, S.W.; Degrado, S.J.; Shu, M.; Klapp, J.M.; Cai, S.J.; Blake, A.; Chan, W.W.; Pasternak, A.; Yang, L.; Patchett, A.A.; Smith, R.G.; Chapman, K.T.; Schaeffer, J.M. *Science*, **1998**, *282*, 737.
- [17] Rivier, J.E.; Hoeger, C.; Erchegeyi, J.; Gulyas, J.; DeBoard, R.; Craig, A.G.; Koerber, S.C.; Wenger, S.; Waser, B.; Schaer, J.C.; Reubi, J.C. *J. Med. Chem.*, **2001**, *44*, 2238.
- [18] Yang, L.; Berk, S.C.; Rohrer, S.P.; Mosley, R.T.; Guo, L.; Underwood, D.J.; Arison, B.H.; Birzin, E.T.; Hayes, E.C.; Mitra, S.W.; Parmar, R.M.; Cheng, K.; Wu, T.J.; Butler, B.S.; Foor, F.; Pasternak, A.; Pan, Y.; Silva, M.; Freidinger, R.M.; Smith, R.G.; Chapman, K.; Schaeffer, J.M.; Patchett, A.A. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 10836.
- [19] Reubi, J.C.; Schaer, J.C.; Wenger, S.; Hoeger, C.; Erchegeyi, J.; Waser, B.; Rivier, J. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 13973.
- [20] Liu, S.; Tang, C.; Ho, B.; Ankersen, M.; Stidsen, C.E.; Crider, A.M. *J. Med. Chem.*, **1998**, *41*, 4693.
- [21] Gilon, C.; Huenges, M.; Matha, B.; Gellerman, G.; Hornik, V.; Afargan, M.; Amitay, O.; Ziv, O.; Feller, E.; Gamliel, A.; Shohat, D.; Wanger, M.; Arad, O.; Kessler, H. *J. Med. Chem.*, **1998**, *41*, 919.
- [22] Pfeiffer, M.; Koch, T.; Schroder, H.; Klutzny, M.; Kirscht, S.; Kreienkamp, H.J.; Holtt, V.; Schulz, S. *J. Biol. Chem.*, **2001**, *276*, 14027.
- [23] Reubi, J.C.; Eisenwiener, K.P.; Rink, H.; Waser, B.; Macke, H.R. *Eur. J. Pharmacol.*, **2002**, *456*, 45.
- [24] Lewis, I.; Bauer, W.; Albert, R.; Chandramouli, N.; Pless, J.; Weckbecker, G.; Bruns, C. *J. Med. Chem.*, **2003**, *46*, 2334.
- [25] Hofland, L.J.; Lamberts, S.W. *Endocr. Rev.*, **2003**, *24*, 28.
- [26] Buchsbaum, D.J. *Semin. Nucl. Med.*, **2004**, *34*, 32.
- [27] Pawlikowski, M.; Melen-Mucha, G. *Neuro. Endocrinol. Lett.*, **2003**, *24*, 21.
- [28] ten Bokum, A.M.; Hofland, L.J.; van Hagen, P.M. *Eur. Cytokine Netw.*, **2000**, *11*, 161.
- [29] Paran, D.; Paran, H. *Curr. Opin. Investig. Drugs*, **2003**, *4*, 578.
- [30] Firestein, G.S. *Nature*, **2003**, *423*, 356.
- [31] Sakane, T.; Suzuki, N. *Clin. Exp. Rheumatol.*, **1998**, *16*, 745.
- [32] Fioravanti, A.; Govoni, M.; La Montagna, G.; Perpignano, G.; Tirri, G.; Trotta, F.; Bogliolo, A.; Ciocci, A.; Mauceri, M.T.; Marcolongo, R. *Drugs Exp. Clin. Res.*, **1995**, *21*, 97.
- [33] Grasland, A.; Pouchot, J.; Vinceneux, P.; Ruszniewski, P. *Arthritis Rheum.*, **2002**, *46*, 277.
- [34] Paran, D.; Elkayam, O.; Mayo, A.; Paran, H.; Amit, M.; Yaron, M.; Caspi, D. *Ann. Rheum. Dis.*, **2001**, *60*, 888.
- [35] Matucci-Cerinic, M.; Borrelli, F.; Generini, S.; Cantelmo, A.; Marcucci, I.; Martelli, F.; Romagnoli, P.; Bacci, S.; Conz, A.; Marinelli, P.; et al. *Arthritis Rheum*, **1995**, *38*, 1687.
- [36] Kurnatowska, I.; Pawlikowski, M. *Neuroendocrinol. Lett.*, **2000**, *21*, 121.
- [37] Takeba, Y.; Suzuki, N.; Takeno, M.; Asai, T.; Tsuboi, S.; Hoshino, T.; Sakane, T. *Arthritis Rheum.*, **1997**, *40*, 2128.
- [38] Elhassan, A.M.; Lindgren, J.U.; Hultenby, K.; Adem, A. *Rheumatol. Int.*, **2001**, *20*, 186.
- [39] Chatterjee, S.; Mbaye, A.; De Man, J.G.; Van Marck, E.A. *Trends Parasitol.*, **2002**, *18*, 295.
- [40] Chatterjee, S.; Van Marck, E. *Trop. Med. Int. Health*, **2001**, *6*, 578.
- [41] Weinstock, J.V.; Elliott, D. *Eur. J. Endocrinol.*, **2000**, *143* (Suppl. 1), S15.
- [42] Elliott, D.E.; Metwali, A.; Blum, A.M.; Sandor, M.; Lynch, R.; Weinstock, J.V. *J. Immunol.*, **1994**, *153*, 1180.
- [43] Robinson, P.; White, A.C.; Lewis, D.E.; Thornby, J.; David, E.; Weinstock, J. *Infect Immun.*, **2002**, *70*, 4534.
- [44] Elliott, D.E.; Blum, A.M.; Li, J.; Metwali, A.; Weinstock, J.V. *J. Immunol.*, **1998**, *160*, 3997.
- [45] Krassas, G.E.; Heufelder, A.E. *Eur. J. Endocrinol.*, **2001**, *144*, 311.
- [46] Pasquali, D.; Notaro, A.; Bonavolonta, G.; Vassallo, P.; Bellastella, A.; Sinisi, A.A. *J. Clin. Endocrinol. Metab.*, **2002**, *87*, 5125.
- [47] Pasquali, D.; Vassallo, P.; Esposito, D.; Bonavolonta, G.; Bellastella, A.; Sinisi, A.A. *J. Mol. Endocrinol.*, **2000**, *25*, 63.
- [48] Lamrani, A.; Tulliez, M.; Chauvelot-Moachon, L.; Chaussade, S.; Mauprivez, C.; Hagnere, A.M.; Vidon, N. *Aliment Pharmacol. Ther.*, **1999**, *13*, 583.
- [49] Chowers, Y.; Cahalon, L.; Lahav, M.; Schor, H.; Tal, R.; Bar-Meir, S.; Levite, M. *J. Immunol.*, **2000**, *165*, 2955.
- [50] Yamamoto, H.; Morise, K.; Kusugami, K.; Furusawa, A.; Konagaya, T.; Nishio, Y.; Kaneko, H.; Uchida, K.; Nagai, H.; Mitsuma, T.; Nagura, H. *J. Gastroenterol.*, **1996**, *31*, 525.
- [51] Reubi, J.C.; Laissue, J.; Waser, B.; Horisberger, U.; Schaer, J.C. *Ann. N.Y. Acad. Sci.*, **1994**, *733*, 122.
- [52] Reubi, J.C.; Mazzucchelli, L.; Laissue, J.A. *Gastroenterology*, **1994**, *106*, 951.
- [53] van Bergeijk, J.D.; Wilson, J.H.; Nielsen, O.H.; von Tirpitz, C.; Karvonen, A.L.; Lygren, I.; Radler, A.; Waldum, H.L.; Mulder, C.J.; Friis, S.; Tefera, S.; Hoogkamer, J.F. *Eur. J. Gastroenterol. Hepatol.*, **2002**, *14*, 243.
- [54] Cavallini, G.; Frulloni, L. *Dig. Liver Dis.*, **2001**, *33*, 192.
- [55] Arvanitidis, D.; Hatzipanayiotis, J.; Koutsounopoulos, G.; Frangou, E. *Hepatogastroenterology*, **1998**, *45*, 248.
- [56] Uhl, W.; Anghelacopoulos, S.E.; Friess, H.; Buchler, M.W. *Digestion*, **1999**, *60* (Suppl. 2), 23.
- [57] Levy, P.; Hastier, P.; Arotcarena, R.; Bartolie, E.; Bougeard-Julien, M.; Blumberg, J.; O'Toole, D.; Ruszniewski, P. *Pancreatology*, **2004**, *4*, 229.
- [58] Karalis, K.; Mastorakos, G.; Chrousos, G.P.; Tolis, G. *J. Clin. Invest.*, **1994**, *93*, 2000.
- [59] Kurnatowska, I.; Pawlikowski, M. *Neuroimmunomodulation*, **2001**, *9*, 119.
- [60] Ferone, D.; van Hagen, P.M.; Pivonello, R.; Colao, A.; Lamberts, S.W.; Hofland, L.J. *Eur. J. Endocrinol.*, **2000**, *143* (Suppl. 1), S27.
- [61] Ferone, D.; Pivonello, R.; Van Hagen, P.M.; Dalm, V.A.; Lichtenauer-Kaligis, E.G.; Waaijers, M.; Van Koetsveld, P.M.; Mooy, D.M.; Colao, A.; Minuto, F.; Lamberts, S.W.; Hofland, L.J. *Am. J. Physiol. Endocrinol. Metab.*, **2002**, *283*, E1056.
- [62] Ferone, D.; Hofland, L.J.; Colao, A.; Lamberts, S.W.; van Hagen, P.M. *Ann. Oncol.*, **2001**, *12* (Suppl. 2), S125.
- [63] Ferone, D.; van Hagen, M.P.; Kwekkeboom, D.J.; van Koetsveld, P.M.; Mooy, D.M.; Lichtenauer-Kaligis, E.; Schonbrunn, A.; Colao, A.; Lamberts, S.W.; Hofland, L.J. *J. Clin. Endocrinol. Metab.*, **2000**, *85*, 1719.
- [64] Ferone, D.; van Hagen, P.M.; Colao, A.; Annunziato, L.; Lamberts, S.W.; Hofland, L.J. *Ann. Med.*, **1999**, *31* (Suppl. 2), 28.

- [65] Ferone, D.; van Hagen, P.M.; van Koetsveld, P.M.; Zuijderwijk, J.; Mooy, D.M.; Lichtenauer-Kaligis, E.G.; Colao, A.; Bogers, A.J.; Lombardi, G.; Lamberts, S.W.; Hofland, L.J. *Endocrinology*, **1999**, *140*, 373.
- [66] Solomou, K.; Ritter, M.A.; Palmer, D.B. *Eur. J. Immunol.*, **2002**, *32*, 1550.
- [67] Krantic, S. *Peptides*, **2000**, *21*, 1941.
- [68] Blum, A.M.; Elliott, D.E.; Metwali, A.; Li, J.; Qadir, K.; Weinstock, J.V. *J. Immunol.*, **1998**, *161*, 6316.
- [69] Lichtenauer-Kaligis, E.G.; van Hagen, P.M.; Lamberts, S.W.; Hofland, L.J. *Eur. J. Endocrinol.*, **2000**, *143* (Suppl. 1), S21.
- [70] Ghamrawy, C.E.; Rabourdin-Combe, C.; Krantic, S. *Peptides*, **1999**, *20*, 305.
- [71] ten Bokum, A.M.; Lichtenauer-Kaligis, E.G.; Melief, M.J.; van Koetsveld, P.M.; Bruns, C.; van Hagen, P.M.; Hofland, L.J.; Lamberts, S.W.; Hazenberg, M.P. *J. Endocrinol.*, **1999**, *161*, 167.
- [72] Oomen, S.P.; Hofland, L.J.; van Hagen, P.M.; Lamberts, S.W.; Touw, I.P. *Eur. J. Endocrinol.*, **2000**, *143* (Suppl. 1), S9.
- [73] Dalm, V.A.; van Hagen, P.M.; van Koetsveld, P.M.; Achilefu, S.; Houtsmuller, A.B.; Pols, D.H.; van der Lely, A.J.; Lamberts, S.W.; Hofland, L.J. *Am. J. Physiol. Endocrinol. Metab.*, **2003**, *285*, E344.
- [74] Peluso, G.; Petillo, O.; Melone, M.A.; Mazzarella, G.; Ranieri, M.; Tajana, G.F. *Neuropeptides*, **1996**, *30*, 443.
- [75] Levite, M.; Chowder, Y. *Ann. Oncol.*, **2001**, *12* (Suppl 2), S19.
- [76] Perego, C.; Lattuada, D.; Casnici, C.; Gatti, S.; Orsenigo, R.; Panagiotis, S.; Franco, P.; Marelli, O. *Int. J. Immunopharmacol.*, **1998**, *20*, 479.
- [77] Levite, M. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 12544.
- [78] Lattuada, D.; Casnici, C.; Venuto, A.; Marelli, O. *J. Neuroimmunol.*, **2002**, *133*, 211.
- [79] van Hagen, P.M. *Metabolism*, **1996**, *45*, 86.
- [80] Vanhagen, P.M.; Markusse, H.M.; Lamberts, S.W.; Kwekkeboom, D.J.; Reubi, J.C.; Krenning, E.P. *Arthritis Rheum.*, **1994**, *37*, 1521.
- [81] Ten Bokum, A.M.; Rosmalen, J.G.; Hofland, L.J.; Krenning, E.P.; Van Hagen, P.M.; Breeman, W.A. *Nucl. Med. Commun.*, **2002**, *23*, 1009.
- [82] Neumann, I.; Mirzaei, S.; Birck, R.; Osinger, K.; Waldherr, R.; Kohn, H.D.; Meisl, F.T. *Rheumatology (Oxford)*, **2004**, *43*, 195.
- [83] Krassas, G.E.; Kahaly, G.J. *Eur. J. Endocrinol.*, **1999**, *140*, 373.
- [84] Lahlou, H.; Guillermet, J.; Hortala, M.; Vernejoul, F.; Pyronnet, S.; Bousquet, C.; Susini, C. *Ann. N.Y. Acad. Sci.*, **2004**, *1014*, 121.
- [85] Minoos, P.; Zadeh, M.M.; Rottapel, R.; Lebrun, J.J.; Ali, S. *Blood*, **2004**, *103*, 1398.
- [86] Cordelier, P.; Esteve, J.P.; Bousquet, C.; Delesque, N.; O'Carroll, A.M.; Schally, A.V.; Vaysse, N.; Susini, C.; Buscail, L. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 9343.
- [87] Florio, T.; Yao, H.; Carey, K.D.; Dillon, T.J.; Stork, P.J. *Mol. Endocrinol.*, **1999**, *13*, 24.
- [88] Guillermet, J.; Saint-Laurent, N.; Rochaix, P.; Cuvillier, O.; Levade, T.; Schally, A.V.; Pradayrol, L.; Buscail, L.; Susini, C.; Bousquet, C. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 155.
- [89] Liu, D.; Martino, G.; Thangaraju, M.; Sharma, M.; Halwani, F.; Shen, S.H.; Patel, Y.C.; Srikant, C.B. *J. Biol. Chem.*, **2000**, *275*, 9244.
- [90] Sharma, K.; Patel, Y.C.; Srikant, C.B. *Mol. Endocrinol.*, **1996**, *10*, 1688.
- [91] Woltering, E.A. *Cancer Biother. Radiopharm.*, **2003**, *18*, 601.
- [92] Lawnicka, H.; Stepien, H.; Wyczolkowska, J.; Kolago, B.; Kunert-Radek, J.; Komorowski, J. *Biochem. Biophys. Res. Commun.*, **2000**, *268*, 567.
- [93] Florio, T.; Morini, M.; Villa, V.; Arena, S.; Corsaro, A.; Thellung, S.; Culler, M.D.; Pfeffer, U.; Noonan, D.M.; Schettini, G.; Albini, A. *Endocrinology*, **2003**, *144*, 1574.
- [94] Hatoum, O.A.; Binion, D.G.; Otterson, M.F.; Gutterman, D.D. *Gastroenterology*, **2003**, *125*, 58.
- [95] Talme, T.; Ivanoff, J.; Hagglund, M.; Van Neerven, R.J.; Ivanoff, A.; Sundqvist, K.G. *Clin. Exp. Immunol.*, **2001**, *125*, 71.
- [96] Buchan, A.M.; Lin, C.Y.; Choi, J.; Barber, D.L. *Am. J. Physiol. Endocrinol. Metab.*, **2002**, *277*, 28431.
- [97] Benali, N.; Cordelier, P.; Calise, D.; Pages, P.; Rochaix, P.; Nagy, A.; Esteve, J.P.; Pour, P.M.; Schally, A.V.; Vaysse, N.; Susini, C.; Buscail, L. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 9180.
- [98] Dasgupta, P. *Pharmacol. Ther.*, **2004**, *102*, 61.