Review of Literature
REVIEW OF LITERATURE:

The somatostatinergic system has proved to be one of the best models in neuropeptide biology. Originally characterized as a hypothalamic regulator of growth hormone (GH) secretion, somatostatin also modulates the secretion of multiple pituitary, pancreatic and GI hormones like TSH, insulin and glucagon and gastric acid (Brazeau et al., 1973; Reichlin, 1995). Somatostatin also functions as a neurotransmitter, modulating locomotor activity and cognitive functions. In recent years, attention has focused on its role in the progression and control of neoplastic disease as well as its function in certain areas of the CNS like the cortex, hippocampus and stratum (Chesslet et al., 1995). Disorders in somatostatin metabolism have been proposed to contribute to pathogenesis of Alzheimer’s disease, epilepsy, Parkinson’s syndrome and GI motility disorders (Gilles, 1997). On a more basic level, studies on somatostatin metabolism have unified diverse concepts in intracellular signal transduction and eukaryotic gene expression (Montminy et al., 1995).

1. CELLULAR BIOSYNTHESIS AND PROCESSING OF SOMATOSTATIN:

The tetradecapeptide, somatostatin, is widely distributed in the central nervous system and the gastrointestinal system. The ubiquitous distribution of this hormone indicates its involvement in multiple biological functions. All vertebrates and primitive invertebrates, contain immunoreactive somatostatin. This suggests that the molecule and its controlling gene(s) evolved before the appearance of differentiated cell-cell and nerve-cell communication. The evolutionary paths of mammals and fish are thought to have diverged at least 400 million years ago. The fact that the phenotype of somatostatin-14 is so well conserved, suggests that throughout evolution, the specific configuration of somatostatin 14 has endowed a selective advantage on the animal organism (Reichlin, 1983).

There are predominantly two biologically active forms of somatostatin: somatostatin-14 and somatostatin-28.
Somatostatin-14:
Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

Somatostatin-28:
Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-somatostatin-14

In mammals these two products are generated by endoproteolytic processing of the prohormone prosomatostatin, which is in turn generated from a 116 amino acid precursor called preprosomatostatin (Reichlin, 1983). The preprohormone is synthesized on the endoplasmic reticulum of neurons cells and translocated to the Golgi system, under the vectorial guidance of a leader sequence which is then cleaved off to generate the active prohormone, 92 amino acids in length, called prosomatostatin (ProSS). The processing of mammalian proSS is tissue and organ specific and results in the generation of four peptides apart from somatostatin-14 and somatostatin-28 (Fig. I). These do not contain the tetradecapeptide structure, but are derived from the somatostatin precursor preprosomatostatin. The biological roles and functions of these peptides remain unknown (Benoit et al., 1990). The mammalian endoproteases like Furin, PACE4, PC1, PC2 are believed to mediate endoproteolysis of prosomatostatin (Patel et al., 1995).

Cloning and sequencing of several cDNA's encoding preprosomatostatin in various species, have shown that mammalian preprosomatostatin is the product of a single gene (Patel et al., 1995). In anglerfish, however, at least two different genes code for preprosomatostatin. Direct or indirect evidence have also been provided for the existence of two genes in catfish, salmon and flounder (Severino et al., 1990).

2. AGONIST ANALOGUES OF SOMATOSTATIN: STRUCTURE-ACTIVITY RELATIONSHIP:
The multiple physiological and pharmacological activities of somatostatin has led to intense research focused on the therapeutic potential of this peptide. However, the
Fig. 1 Schematic diagram summarizing the processing of mammalian pre-proSS. The "central peptide" shown with the dashed box is only hypothetical and has been found in any tissue.
Adapted from Benoit et al. (1990) Metabolism 39 (Suppl 1), 22-25.
Pre-proSS: Preprosomatostatin
pro-SS: prosomatostatin
SS-14: somatostatin-14
SS-28: somatostatin-28
therapeutic utility of somatostatin remains limited by its short half life in serum. Systematic structure activity relationship (SAR) studies, along with NMR and various modeling studies, have led to the design and synthesis of much smaller somatostatin analogs which retain the biological activity of the parent peptide and are resistant to enzymatic degradation.

Early SAR studies established that the Ala\(^1\) Gly\(^2\) residues and the disulfide bonds are not essential for bioactivity. The oxidized and reduced forms of synthetic somatostatin, as well as (Des-Ala\(^1\), des-Gly\(^2\)) somatostatin were equipotent in inhibiting growth hormone secretion \textit{in vitro} and \textit{in vivo}. Replacements of Lys\(^4\) by Arg, Phe, Phe(F5) or Phe(p-NH\(_2\)) residues; Asn\(^5\) by Ala or D-Trp; Phe\(^7\) by Tyr; Phe\(^{11}\) by Phe(p-I) or Nal resulted in compounds equipotent to somatostatin whereas replacement of Trp\(^8\) by D-Trp, D-Trp(5-F), D-Trp(6-F), D-Trp(5-Br) generated compounds more potent than the parent peptide. Deletion of the carboxyl group, or its replacement with an ethylamide group also resulted in compounds equipotent to somatostatin. An equally important finding useful for designing small peptides, emerged from deletion studies. Compounds lacking Lys\(^4\) and Asn\(^5\) were found to retain significant biological activity, while compounds lacking Phe\(^6\) Trp\(^8\), Lys\(^9\), Thr\(^{10}\), Phe\(^{11}\), Thr\(^{11}\) were relatively poor agonists of somatostatin.

NMR studies seem to suggest that the most probable conformation of the cyclic part of naturally-occurring somatostatin was that of an extended antiparallel \(\beta\)-sheet with residues Trp\(^8\)-Lys\(^9\) at the corners of a type II \(\beta\)-turn (Melacini \textit{et al}., 1997). Previous studies have demonstrated that the entire native hormone is not essential for the full activity spectrum (Bauer \textit{et al}., 1982). The essential pharmacophore of somatostatin comprises of the following fragment:

\[
Phe^{7}\text{-Trp}^{8}\text{-Lys}^{9}\text{-Thr}^{10}
\]

These SAR studies along with NMR and modeling studies, led to the design and synthesis of cyclic hexapeptides, which were equipotent or up to 50-fold more potent in at least some test systems. A conformationally adequate surrogate for the full hormone sequence was chosen to be the following cyclic hexapeptide:
By means of both physical and computer-assisted model building the N-terminal and C-terminal residues were added, as well as the central pharmacophore was modified to generate extremely potent peptides with prolonged action. The total number of somatostatin analogs synthesized so far is more than 450. Some of the most potent, stable and extensively studied agonist analogs of somatostatin are:

**RC-160**: DPhe-Cys-Tyr-DTrp-Lys-Val-Cys-Trp-NH₂

**Octreotide**: H-DPhe-Cys-Phe-DTrp-Lys-Thr-Cys-Thr(ol)

**BIM 23014**: H-DNal-Cys-Tyr-DTrp-Lys-Val-Cys-Thr-NH₂

**MK678**: c[N-Me-Ala-Tyr-DTrp-Lys-Val-Phe]

The three dimensional conformation of these cyclic octapeptides resembles the native peptide (Fig. II). NMR studies involving the structure of RC-160 and Re-RC-160, reveal that RC-160 maintains the same backbone conformation as the parent somatostatin, with an antiparallel β-sheet and a tight type II' β-turn centered at D-Trp⁴-Lys⁵ (Varnum et al., 1994).

The somatostatin analogs like octreotide and RC-160 are potent inhibitors of GH secretion in vivo. However, they possessed lower activity for the inhibition of insulin and glucagon. Such differential activity of these peptides is due to the existence of multiple somatostatin receptor subtypes having selective affinities for structural analogs of somatostatin, and with a selective expression in different organ systems.

One of the first examples of a somatostatin analog, in which the anti-tumor activity was divorced from the GH-release inhibition function of somatostatin was TT2-32 (Keri et al., 1996; Szegedi et al., 1999). It displayed very high anti-proliferative
Fig. II A possible low energy conformation of octreotide (A) with an identical view of the corresponding fragment of somatostatin-14 (B).

Adapted from Bauer et al. (1982) Life Sci. 31, 1133-1140.
activity, but very low anti-secretory (inhibition of GH secretion) activity \textit{in vitro} and \textit{in vivo}.

\textbf{TT2-32: D-Phe-Cys-Tyr-DTrp-Lys-Cys-Thr-NH$_2$}

The conformation of TT2-32 clearly indicates that the peptide backbone behaves very differently from that observed in GH active somatostatin analogs like RC-160. Molecular dynamics on TT2-32, reveal the presence of one \(\gamma\)-turn around Lys$^9$ and possibly another around D-Trp$^8$ (Jaspers \textit{et al}., 1994).

The actions of somatostatin are mediated by specific, high affinity membrane G-protein coupled receptors on target cells. The cloning of individual somatostatin receptor subtypes, has led to the design and synthesis of receptor subtype specific analogs. Raynor \textit{et al}., (1993) have designed and synthesized biologically active linear somatostatin analogs with subtype specificity for SSTR2. Analogous to TT2-32, the ability of BIM23066 to inhibit growth hormone secretion is very low.

\textbf{BIM23066 : NH$_2$-D-Phe-p-NO$_2$-Phe-Tyr-DTrp-Lys-Val-Phe-Thr-NH$_2$}

Extensive studies involving somatostatin structure have yielded agonists of varying potency, however, for a very long time no somatostatin antagonist had been described. A landmark achievement in somatostatin biology has been the synthesis of somatostatin antagonists which reverse somatostatin mediated inhibition of cAMP in rat sommatotroph cells (Bass \textit{et al}., 1996). These are octapeptides containing a disulfide bond between D-Cys$^2$ and L-Cys$^7$.

\textbf{Ac-4-NO$_2$-Phe-D-Cys-Tyr-DTrp-Lys-Thr-Cys-D-Tyr-NH$_2$}

It seems the presence of D-Cys at the 2 position is essential for antagonist activity. Identical peptides containing L-Cys at the 2 position manifest agonistic properties.
Similarly, Liapakis et al. (1996) have been able to synthesize the first somatostatin analog CH275 which is highly specific for the somatostatin receptor subtype 1 (SSTR1).

CH 275: Cys-Lys-Phe-Phe-Trp-IAMP-Thr-Phe-Thr-Ser-Cys-OH

The somatostatin scaffold has been of great utility in the design of agonists and antagonists to other G-protein coupled neuropeptides. Potent antagonists to oxytocin, neuromedin B and substance P have been synthesized using somatostatin agonists as the peptide backbone (Hruby, 1987; Orbuch et al., 1993; Hirschmann et al., 1996).

The development of specific agonists and antagonists of somatostatin, will provide new tools for drug design. The use of the somatostatin backbone to design potent and selective analogs to other neuropeptides, will expand the pharmacological utility of somatostatin in disease, and improve its therapeutic index.

4. SOMATOSTATIN RECEPTORS:
The physiological actions of somatostatin are mediated by high affinity membrane bound G-protein coupled receptors on target cells (Patel et al., 1997; Lamberts et al., 1995). Five receptor subtypes, with varying affinities for synthetic agonists, such as octreotide, MK 678 etc., have been identified and characterized in the CNS as well as the endocrine tissues. Somatostatin receptors are coupled via guanine nucleotide binding proteins (G-proteins) to multiple cellular effector systems and are known to mediate the inhibition of adenyl cyclase activity, and Na⁺/H⁺ exchanger activity, via pertussis toxin insensitive G-proteins (Patel et al., 1997; Hou et al., 1994). The recent cloning and functional characterization of five somatostatin receptor subtypes has been a major breakthrough in understanding their functional and biochemical properties (Reisine et al., 1995).
The human somatostatin receptor subtypes (designated hSSTR1-hSSTR5) vary in size from 364 amino acids (hSSTR5) to 418 amino acids (hSSTR3), with 39-57% amino acid identity with 105 amino acids being invariant. SSTR1 and SSTR4 show the highest sequence identity. The individual subtypes display a remarkable degree of structural conservation across the species. Thus, there is a 94-98% sequence identity between the human, rat and mouse isoforms of SSTR1; 93-96% sequence identity between human, rat, mouse, porcine and bovine SSTR2; and 88% sequence identity between the rat and human isoforms of SSTR4; SSTR3 and SSTR5 are somewhat less conserved, showing 82-83% sequence identity between the human and rodent homologues. The nearest relatives of the somatostatin receptors are opioid receptors whose δ subtype displays 37% sequence similarity to SSTR1 (Patel et al., 1997).

The somatostatin receptors are glycoproteins. The carbohydrate component of the somatostatin receptor may be involved in promoting high affinity ligand binding. The sequences of the seven putative α-helical membrane-spanning domains are more highly conserved than the extracellular amino terminal and intracellular carboxyl terminus domains (Fig. III). Human somatostatin receptors are encoded by a family of five nonallelic genes located on separate chromosomes (Patel et al., 1997). Four of the genes are intronless, the exception being SSTR2, which gives rise to spliced variants SSTR2A and SSTR2B, which differ in the size and sequence of the C-terminal domain (Fig. III). Thus, the functional diversity of the somatostatin receptor family may result from the expression of multiple types as well as from alternative splicing.

4.1 Pharmacological Properties of Cloned Receptor subtypes:

The cloning and expression of somatostatin receptor subtypes in a variety of cell lines (having few endogenous somatostatin receptors), has facilitated pharmacological studies of individual receptors. All the receptor subtypes bind to somatostatin-14 and somatostatin-28 with high affinity (Table 1). All five receptors mediate the inhibition of adenylyl cyclase; this inhibition is sensitive to pertussis toxin (Patel et al., 1997). The binding profiles of short synthetic somatostatin analogs like
Fig. III. Schematic depiction of the seven-transmembrane topology of the human SSTR1-5 receptors. CHO are potential sites for N-linked glycosylation within the amino terminal segment and second extracellular loops (ECL); PO₄ are putative sites for phosphorylation by protein kinase A, protein kinase C and casein kinase. The cysteine residue 12 amino acids downstream from the VIth TM is conserved in SSTR (1,2,4,5) and may be the site of a potential palmitoyl membrane anchor. The YANSCAN PI/VLY sequence in the VIITM is highly conserved in all members of the SSTR family. Residues Asp¹³⁷, Asn¹⁹¹ and Phe²⁸⁷ in TMs III, VI and VII, respectively, of SSTR2A have been proposed to form part of a ligand-binding pocket for octreotide and are shown in closed circles.

TABLE 1. Ligand selectivity of cloned human somatostatin receptors

<table>
<thead>
<tr>
<th>ANALOG</th>
<th>SSTR1</th>
<th>SSTR2</th>
<th>SSTR3</th>
<th>SSTR4</th>
<th>SSTR5</th>
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<tbody>
<tr>
<td>SOM-14</td>
<td>0.1-2.26</td>
<td>0.2-1.3</td>
<td>0.3-1.6</td>
<td>0.3-1.8</td>
<td>0.2-0.9</td>
</tr>
<tr>
<td>SOM-28</td>
<td>0.1-2.2</td>
<td>0.2-4.1</td>
<td>0.3-6.1</td>
<td>0.3-7.9</td>
<td>0.05-0.04</td>
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<td>OCT.</td>
<td>290-1140</td>
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<td>&gt;1000</td>
<td>5.6-32</td>
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<td>BIM.</td>
<td>500-2330</td>
<td>0.5-1.8</td>
<td>43-107</td>
<td>66-2100</td>
<td>0.6-14</td>
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<td>RC-160</td>
<td>&gt;1000</td>
<td>5.4</td>
<td>31</td>
<td>45</td>
<td>0.7</td>
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<tr>
<td>MK-678</td>
<td>&gt;1000</td>
<td>0.1-1.5</td>
<td>27-36</td>
<td>127-&gt;1000</td>
<td>2-23</td>
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<tr>
<td>BIM-23052</td>
<td>6.3-100</td>
<td>10-13.5</td>
<td>2.1-5.6</td>
<td>16-141</td>
<td>1.2-7.3</td>
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<tr>
<td>BIM-23056</td>
<td>110-1000</td>
<td>132-&gt;1000</td>
<td>10.8-177</td>
<td>17-234</td>
<td>5.7-14.1</td>
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<tr>
<td>L-362855</td>
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<td>1</td>
<td>6.2</td>
<td>63-&gt;1000</td>
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<td>CH-275</td>
<td>3.2-4.3</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>4.3-874</td>
<td>&gt;1000</td>
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</table>


SOM-14 : SOMATOSTATIN-14
SOM-28 : SOMATOSTATIN-28
OCT.  : OCTROTIDE
BIM  : BIM 23014
The octapeptide analogs of somatostatin, octreotide and RC-160 display especially high affinity binding to SSTR2 and SSTR5. The receptor subtype SSTR2 mediates the anti-proliferative effect of somatostatin analogs in vitro, by activation of a phosphotyrosine phosphatase (Table 2B) (Buscail et al., 1994, 1995; Douzeich et al., 1999). The role of SSTR2 in the negative control of cell proliferation is further strengthened by the presence of this subtype in human breast cancer cells, SCLC cells and pancreatic cancer cells which respond in vitro and in vivo to somatostatin analogs (Hofland et al., 1994; Tang et al., 1998; Feindt et al., 1999). The stimulation of SSTR1 has been reported to also mediate the anti-proliferative activity of somatostatin.

The apoptosis of cancer cells (in response to octreotide therapy) seems to be mediated uniquely via the SSTR3 subtype, on target cells (Srikant, 1995; Sharma et al., 1996). In mitogen-activated JURKAT T-cells the SSTR3 receptor isotype mediates the stimulatory action of somatostatin on IL-2 secretion and cell proliferation. The linear somatostatin analogue BIM23056 appears to bind selectively to SSTR3, and is being used to elucidate functional properties of this receptor subtype.

**BIM-23056**: D-Phe-Phe-Tyr-DTrp-Lys-Val-Phe-Nal-NH₂

The ligand binding properties of SSTR5 are unique in the respect that its affinity for somatostatin-28 is 4-6 fold higher than somatostatin-14.
TABLE 2A. Characteristics of the cloned subtypes of human somatostatin receptors

<table>
<thead>
<tr>
<th></th>
<th>SSTR1</th>
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<th>SSTR3</th>
<th>SSTR4</th>
<th>SSTR5</th>
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<td>22q13.1</td>
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<td>Inhibition of GH,</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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<td>Gastric Acid and</td>
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<td>Glucagon</td>
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<tr>
<td>Insulin Inhibition</td>
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<td>Amylase Inhibition</td>
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<td>Gastric Smooth</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Muscle Contraction</td>
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TABLE 2B. Characteristics of the cloned subtypes of human somatostatin receptors

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<th>EFFECTOR COUPLING</th>
<th>SSTR1</th>
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<td>TYROSINE PHOSPHATASE ACTIVITY</td>
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<tr>
<td>Ca²⁺ CHANNELS</td>
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<td>Na⁺ / H⁺ EXCHANGER</td>
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<tr>
<td>PHOSPHOLIPASE C / IP₃ ACTIVITY</td>
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<tr>
<td>PHOSPHOLIPASE A2 ACTIVITY</td>
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<td></td>
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</tr>
<tr>
<td>MAP KINASE ACTIVITY</td>
<td>↓</td>
<td></td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>TISSUE DISTRIBUTION</td>
<td>Brain, pituitary, liver, pancreas, kidney</td>
<td>Brain, pituitary, liver, pancreas, kidney</td>
<td>Brain, pituitary, stomach</td>
<td>Brain, stomach, lung, pancreas</td>
<td>Brain, pituitary, stomach</td>
</tr>
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</table>


IP₃: INOSITOL TRIPHOSPHATE

MAP KINASE: MITOGEN-ACTIVATED PROTEIN KINASE
L-362,855: c(Aha-Phe-p-Cl-Phe-D-Trp-Lys-Thr-Phe)
BIM-23052: D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH$_2$

The analogs L-362,855 and BIM23052 bind to SSTR5 at a 1000-fold higher affinity, than other somatostatin receptor subtypes. Studies by Wilkinson et al., (1997) suggest that the peptide BIM23056 is a specific antagonist which binds to SSTR5 receptor whereas Reisine et al. (1995) have demonstrated the selective binding of BIM23056 to SSTR3. The somatostatin analog CH275 has been claimed to be specific for SSTR1 (Liapakis et al., 1996; Chen et al., 1999). However, Patel et al., (1997) demonstrated that CH275, also functions as a selective agonist for SSTR4.

Most of these studies aimed at determining subtype specificity are done using transfected cells, and the results need to be interpreted with caution, keeping in mind the limitations of these systems.

The elucidation of molecular interactions between somatostatin agonists and their receptors was done by molecular modeling. All the somatostatin receptor isoforms are closely related in size and sequence. They display seven transmembrane domain topology and possess a highly conserved sequence motif YANSCANPIIVL Y in the seventh transmembrane domain, which serves as a signature sequence for this receptor family (Patel et al., 1997; Reisine et al., 1995). Identification of the precise determinants for ligand binding to SSTR1 and SSTR2 may be critical for the rational design of peptide and non-peptide ligands which are less pleotropic than the agonists presently available. Fritzpatrick and Vandlen (1994) used the selective SSTR2 agonist MK678 to map the ligand binding domains of murine SSTR2 using site-directed mutagenesis. They reported that the extracellular domains of murine SSTR2, particularly regions encompassing the second and third extracellular loops, were essential for agonist binding as has already been reported for tachykinin and κ-opioid receptors. Kaupman et al. (1995) have clearly proved that Phe$^{295}$ and Asn$^{291}$ are critical for the binding of octapeptides like octreotide to SSTR2. Similarly, Liapakis et al. (1996) observed that SSTR1 exhibited low affinity for several SSTR2 selective ligands like MK678 and octreotide. Their findings revealed that a 4 amino acid sequence FDFV in the transmembrane 7 of murine SSTR2 is essential for high affinity binding of octapeptides (like octreotide) but not for hexapeptides like...
MK678. They also observed that an aromatic residue at position 2 is essential for high affinity binding of hexapeptides to SSTR2. Most interestingly, the high affinity binding of the hexapeptides to SSTR2 is dependent on the presence of Phe (and not Tyr) at position 2. Thus a single hydroxyl group plays a critical role in determining relative binding affinities of hexapeptides to SSTR2.

Molecular modeling studies led to the elucidation of the critical amino acid residues mediating the interaction of peptide analogs with SSTR2 (Liapakis et al., 1996; Kaupman et al., 1995). The three dimensional structure of bacteriorhodopsin, was used as the template for the folding of G-protein receptors, and models for SSTR2 were prepared. These models clearly define a lipophilic cavity for Trp⁴ of octreotide (Fig. IV). A similar binding site has been proposed for aromatic nucleus of serotonin, dopamine and noradrenaline (Hilbert et al., 1993). Another important residue is Tyr¹⁹⁵ in SSTR2 which forms stabilizing π-π interaction with Phe⁷ of somatostatin or Phe³ of octreotide. The ligand binding pocket of SSTR2 is lined with residues Phe²⁸⁷, Asn²⁹¹, Phe³⁰⁵, which are involved in lipophilic interactions with the Phe 6-Phe11 assembly of somatostatin and the disulfide bridge of octreotide (Kaupman et al., 1995).

The overall model that emerges from these studies suggests a binding domain for somatostatin and its analogs made up of hydrophobic residues within the transmembrane III-VII, with a potential contribution by extracellular loops, and is consistent with other peptide binding G-protein coupled receptors like neurokinin A and angiotensin II, which interact with residues in both extracellular loops and the transmembrane domains (Schwartz et al., 1996).

5. SOMATOSTATIN AND THE REGULATION OF GH SECRETION:

The hypothalamus plays a critical role in communication between the brain and the body, as it is the key site where information from higher centers is integrated and transduced to the peripheral tissues. The somatostatin neurons in the periventricular nucleus are involved in the regulation of GH secretion (Fig. V). Somatostatin-14 and
Fig. IV  Schematic representation of the interaction between SSTR2 and octreotide. The circles represent the seven transmembrane helices.

Adapted from Kaupman et al. (1995) The EMBO Journal 14, 727-735.
Fig. V Basic elements of the neuroendocrine system and its regulation of growth hormone secretion.

somatostatin-28 inhibit GH secretion, to a similar extent. GH secretion is also regulated at the hypothalamic level by cholinergic and adrenergic pathways originating outside the hypothalamus (Fig. VI) (Reichlin, 1995).

The secretion of growth hormone (GH) is markedly pulsatile in nature in both man and animals. Several lines of evidence indicate that the episodic pattern of GH release from the pituitary gland is generated by an exquisite interplay between two hypothalamic neuropeptides, growth hormone releasing hormone (GHRH) and the inhibitory hormone somatostatin (Gilles, 1997).

Somatostatin acts at somatotroph cells in the anterior pituitary gland via its G-protein coupled transmembrane domain receptors, to reduce cAMP formation, and ultimately to inhibit the secretion, but not the synthesis of GH. The action is counter-balanced by GHRH, which is also essential for determining the somatotroph cell number (Gilles, 1997).

In every mammalian species studied so far, spontaneous episodes of GH secretion occurs several times over a 24 hour period. In the male rat, GH pulses are characterized by high amplitude peaks occurring at approximately 3 hours, which in turn depend on patterns of somatostatin and GHRH secretion into the hypothalamo-hypophyseal vessels. Indirect evidence suggests that low baseline GH levels occur because somatostatin is secreted in pulses which are 180° out of phase with those of GHRH and GH (Gilles, 1997) (Fig. VII).

Initial studies with somatostatin analogs favor a role for SSTR2, in mediating the inhibitory effect on somatostatin on GH secretion. However, Shimon et al., (1997) have shown that both the somatostatin receptor subtypes SSTR2 and SSTR5 are involved in GH regulation in primary somatotroph adenoma cells, whereas SSTR5 exclusively regulates prolactin secretion from prolactinoma cells. Hence, somatostatin analogs like octreotide and RC-160 with increased subtype specificity for SSTR2 or SSTR5 seem to be more effective in the treatment of GH- or prolactin secreting pituitary adenomas. However, in a SSTR2 knockout mouse generated by Zheng et al. (1997) SSTR2 receptors in GHRH neurons were found to be responsible
Fig. VI Schematic representation of the interactions and feedback loops within the hypothalmo-pituitary-growth hormone axis. Broken lines represent feedback loops (positive or negative, as indicated). Ach: acetylcholine; DA: dopamine; GH: growth hormone; GHRH: growth hormone releasing hormone; 5-HT: 5-hydroxytryptamine; IGF/II: insulin-like growth factor I/II; NA: noradrenaline; SRIH: somatostatin

Fig. VII. Hypothesis for the hypothalamic control of the pulsatile secretion of growth hormone (GH) in the male rat.

SRIH: somatostatin.

GHRH: growth hormone releasing hormone.

for mediating GH-induced feedback inhibition of GH. The knockout mice grew normally and appeared healthy till 15 months of age, excluding an essential role for SSTR2 in embroyogenesis or postnatal growth and development. Although further work is required on this model, the findings so far do not support a preferential role of SSTR2 in pituitary or other peripheral targets in the rodent system.

The cloning of the somatostatin receptor family represents an important beginning in unraveling the myriad physiological functions of somatostatin. Some of the biological activities of somatostatin, such as the inhibition of GH secretion from normal tissues and GH secreting tumors, suppression of the basal and stimulated secretion from exocrine and endocrine cells, and inhibition of cell proliferation are the targets for specific therapeutic agents (Coy et al., 1995). However, studies of somatostatin action have been hampered by lack of a detailed understanding of its receptors; their number, sequences, tissue distribution and biochemical and pharmacological properties. The cloning of individual receptor subtypes has made possible the investigation of the function of each subtype in mediating the multiple effects of somatostatin in the brain and the in the periphery (Hirota et al., 1998). Furthermore, the availability of cloned receptors will also facilitate the identification of receptor subtype-selective agonists and antagonists, thereby providing new therapeutic opportunities for the treatment of endocrine, neurodegenerative and oncological disorders.

5.1 Acromegaly:
Acromegaly is a symptomatically disabling condition, resulting almost invariably from a growth hormone (GH) secreting pituitary tumor. It causes disfigurements of the face, hands and feet in adults, in children it causes gigantism (Lamberts et al., 1995). Subcellular mechanisms support the notion of an intrinsic pituitary defect in this disease, with elevated growth hormone (GH) and insulin growth factor (IGF-I) levels that affect the cardiovascular and respiratory system as well as the proliferation of neoplastic cells. Surgery, even with external beam adjuvant therapy is successful in less than 60% of the patients, and is associated with side effects. However, an alternate effective and safe long-term medical treatment is essential for about 35% of the patients in whom the pituitary tumor cannot be removed by trans-sphenoidal
surgery. The recent advent of peptide-based therapy has revolutionized the approach to manage patients. The somatostatin analog, octreotide normalizes GH levels and IGF-I levels in upto 60% of the patients, reduces tumor burden in 90% of the patients (Melmed et al., 1996).

In most patients with acromegaly, a single subcutaneous injection of 50μg of octreotide suppresses circulating GH levels to virtually undetectable levels for 6-8 hours, after which the levels slowly return to normal. Long term therapy of acromegalic patients, with octreotide in a dose of 100 μg subcutaneously two or three times daily resulted in a marked clinical improvement in days. The initially raised levels of circulating IGF-I levels return to normal in most patients (Lamberts et al., 1995).

At present octreotide has to be administered subcutaneously twice of thrice daily. Long acting applications of octreotide and lantreotide have been devised in which the peptide is entrapped within microspheres of DL-lactide-co-glycollide polymer. Preliminary data seems to suggest that this intramuscular depot is well tolerated and controls GH hypersecretion in acromegalis for 28-45 days. Pharmacokinetic profiles on these long-acting sustained release formulations for octreotide (Sandostatin™ LAR) and lantreotide (Somatuline™ LP) (Grass et al., 1995; Johnson et al., 1995) clearly demonstrate that there is a need to modify these systems to improve their efficiency and delivery properties (refer to Introduction, page 2).

The somatostatin analogs RC-160 and BIM23014 are currently under clinical investigation, regarding their activity profile in acromegaly. The effects of RC-160, BIM23014 and octreotide on GH release by cultured cells of human GH-secreting pituitary tumors, as well as in normal rat anterior pituitary cells, has been studied in vitro. RC-160 appeared to be about 500-times more potent than octreotide and BIM23014 in inhibiting GH release in vitro (Hofland et al., 1994).

Apart from acromegaly, somatostatin can also control clinical complications caused by hormone hypersecretion in islet cell tumors, insulinomas, VIPomas, Cushing’s syndrome and carcinoid tumors (Lamberts et al., 1995). The hypersecretion of
hormones, peptides and biogenic amines in carcinoid tumors causes flushing diahorrea, hypoglycemic attacks, peptic ulceration, dehydration etc. Octreotide and lanreotide have become the drugs of choice for the symptomatic treatment of carcinoid syndrome (Lamberts et al., 1995). At doses of 100-200μg two or three times a day they give objective biochemical responses in 40-70% of patients and a significant (50%) tumor size reduction in 4-10% of patients. There is marked improvement in the quality of life of these patients during octreotide therapy; preliminary evidence also suggests prolonged survival. Long acting formulations of both octreotide and lanreotide are in clinical trials.

6. SOMATOSTATIN AND CANCER:

Somatostatin is known to be a potent inhibitor of exocrine and endocrine secretion, as well of tumor cell growth. Somatostatin analogs like octreotide, RC-160, somatuline are an important therapeutic agent in the treatment of acromegaly, tumors of the amine precursor uptake and decarboxylation system (glucagonomas, carcinoid tumors etc.), complications of pancreatic surgery and severe forms of diahorrea. RC-160 and somatuline are in various phases of clinical trials (Pollak and Schally, 1998).

The anti-proliferative activity of somatostatin analogs octreotide, RC-160 and BIM23014 have been demonstrated in several experimental models of mammary, renal, pancreatic and prostate cancers (Weckbecker et al., 1993; Marschke et al., 1999; Rubin et al., 1999; Kourmalis et al., 1998). Somatostatin receptors have been found to be overexpressed on several human neuroendocrine and hormone-secreting tumors. The injection of octreotide tagged to radioactive indium ([111In]-DTPA-octreotide) has been used to successfully image somatostatin receptor-positive tumors. These include primary carcinoids, islet tumors and unexpected distant metastases (Lamberts et al., 1991; Colao et al., 1999). This technique, called as “Somatostatin Receptor Scintigraphy” has also been used to visualize malignant lymphomas, tissues affected by granulomatous diseases and some autoimmune disorders (Krenning et al., 1993). The applications of this technique are manifold. It predicts the somatostatin receptor status of tumors in vivo, and thereby the response of the patient to octreotide therapy. Radiolabeled somatostatin analogs have been also
used for the radiotherapy of inoperable somatostatin-receptor positive tumors. Lastly, radiolabeled somatostatin analogs were shown to be successful in radioguided surgery.

6.1 *In Vitro and in vivo* activity of somatostatin analogs:
The somatostatin analogs RC-160, octreotide and BIM23014 have been extensively tested *in vitro* with regard to their anti-proliferative activity (Lamberts *et al.*, 1995) (Table 3). These analogs also display anti-neoplastic activity in various rodent cancer models, with special emphasis on human tumor xenotransplants in nude mice. The effect of these analogs was determined by recording the change in tumor volume during and after treatment. Only a few studies have addressed the inhibitory effect of somatostatin on carcinogenesis or metastatic spread of tumors (Weckbecker *et al.*, 1993). The somatostatin analogs were administered with daily bolus injections, continuous infusion using mini-osmotic pumps or by entrapping the drug in biodegradable microspheres (Gaztambide and Vasquez, 1999; Rubin *et al.*, 1999).

Frequently, the outcome of somatostatin therapy has been related to the somatostatin receptor status of the tumors being treated. The receptor subtype SSTR2 mediates the anti-proliferative effect of RC-160 *in vitro*, by activation of a phosphotyrosine phosphatase (Buscail *et al.*, 1994, 1995; Douziech *et al.*, 1999). The role of SSTR2 in the negative control of cell proliferation has been further strengthened by the presence of this subtype in human breast cancer, SCLC, neuroblastomas, mesenchymal tumors and pancreatic cancer, which respond *in vitro* and *in vivo* to the effect of somatostatin analogs (Pollak and Schally, 1998; Feindt *et al.*, 1999; Fischer *et al.*, 1998). Kubota *et al.* (1996) have detected the presence of somatostatin receptor subtypes in human endocrine tumors. They found that out of all somatostatin receptor subtypes human SSTR2 shows a high affinity for octreotide (which is used to clinically treat endocrine tumors). Furthermore, they observed that octreotide was effective in the treatment of a patient with glucagonoma in which SSTR2 mRNA was detected, but had no effect in a patient with carcinoid in which SSTR2 was not expressed. Delesque *et al.* (1997), observed that unlike normal exocrine pancreas SSTR2 was not expressed in certain pancreatic cell lines. However, the restoration of SSTR2 expression in these cell lines, by stable transfection with SSTR2 cDNA, resulted in significant reduction of cell growth.

<table>
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<tr>
<th>SNo.</th>
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<th>CANCER TYPE</th>
<th>CELL LINE</th>
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OCT. : OCTREOTIDE
BIM : BIM 23014
SOM. : SOMATOSTATIN
(Delesque et al., 1997). Furthermore, Rochaix et al. (1999) have explored the therapeutic potential of SSTR2 based gene therapy for pancreatic carcinoma. They have observed potent anti-tumor activity after transfection of SSTR2 cDNA in pancreatic carcinoma cells. Hence, the interaction of somatostatin analogs with SSTR2 is a very important factor determining the magnitude of their anti-neoplastic activity. However, opposed to the in vitro situation, where only somatostatin receptor positive tumor cells are sensitive to somatostatin analogs, tumors with and without specific, high affinity somatostatin receptors can both respond in vivo to treatment with somatostatin analogs (Pollak and Schally, 1998). Accordingly, the in vivo anti-tumor activity of somatostatin analogs can either be mediated directly through somatostatin receptors, or indirectly by the inhibition of one or several growth factors (indirect effect), aiding the growth and proliferation of cancer cells.

Several breast cancer models have been shown to express a high density of somatostatin receptors on their cell surface. The growth of the human estrogen dependent breast carcinoma MCF-7 in nude mice was significantly retarded by twice daily injections of octreotide (200μg/kg) and by subrenal capsules of BIM-23014. Similarly, continuous infusion of octreotide at 10μg/kg/hr. in nude mice bearing ZR-75-1 (human breast carcinoma) induced a highly significant retardation of tumor growth over a 4 week treatment period (Weckbecker et al., 1993).

"Indirect effects" may also play an important role in the treatment of mammary cancer using somatostatin analogs. Dimethylbenzanthracene (DMBA)-induced rat tumors are heterogeneous in somatostatin receptor expression but they responded to continuous infusion treatment with octreotide (10μg/kg/hr), in the respect that tumor multiplicity was significantly reduced (Weckbecker et al., 1993). Evidence seems to indicate that somatostatin analogs are more active in the initial phases of tumorogenesis. Liebow et al. (1993) showed that RC-160 reverses the development of malignancies initiated by the local administration of DMBA. Although, somatostatin receptor scintigraphy has already shown that MDA-MB-468 lacks specific high affinity somatostatin receptors, but tumor growth was significantly retarded in vivo by either continuous infusion or bolus injections of octreotide (Weckbecker et al., 1993).
The poor prognosis of pancreatic cancer reflects the inadequacy of current therapies. Studies in different animal models have shown that octreotide can cause significant retardation of human pancreatic adenocarcinomas, as well as rat pancreatic acinar tumors. The somatostatin analog RC-160 was found to inhibit the growth of nitrosamine induced pancreatic cancers in hamsters. Microcapsules of RC-160 significantly inhibited tumor growth in nude mice bearing MIA-PaCa2 tumors. The growth of MIA-PaCa2 tumors was dose dependently inhibited in nude mice by bolus injections of octreotide (Weckbecker et al., 1993). Somatostatin has been also suggested to be useful for therapy of prostate cancer. It was found that the somatostatin analogs significantly inhibited the growth and progression of R-3327 Dunning rat prostate tumors (Weckbecker et al., 1993). It was found that tumor growth was inhibited by these peptides but interruption of treatment resulted in the re-growth of tumors.

The effects of somatostatin analogs on colon cancer have been extensively studied. It has been found that octreotide and RC-160 can inhibit the growth of human colon adenocarcinomas in nude mice. Long term treatment of RC-160 inhibited the growth of liver metastases induced by intrasplenic injection of DHD/K12 colon adenocarcinoma cells into syngenic BDIX rats (Weckbecker et al., 1993). Similarly, human SCLC cell lines NCI-N417, LX-1, NCI-H345, (Weckbecker et al., 1993) human renal adenocarcinoma cell line Caki-I, xenotransplanted in nude mice (Jungwirth et al., 1998) respond well to therapy, involving RC-160, octreotide and BIM23014.

Keri et al. (1996) reported a unique potent tumor selective somatostatin analog TT2-32, which has practically no GH release inhibitory activity in vitro and in vivo. TT2-32 inhibited the proliferation of 20 different cancer cell lines in vitro, in the range of 50-95% and was observed to induce very strong apoptosis. TT2-32 has been tested on 60 various human tumor cell lines, representing different cancers, by the National Cancer Institute, NIH. In contrast, somatostatin analogs like RC-160 and octreotide had practically no effect on these above cell lines. TT2-32 was effective on human tumors xenotransplanted in nude mice especially those of pancreas, prostate, breast,
colon carcinoma, melanoma and sarcoma (Szegedi et al., 1999). TT2-32 is also an extremely potent inhibitor of metastases as studied in vivo in mice, causing a 70% inhibition in metastases. All these data suggest that TT2-32 is an attractive candidate as a potent and selective anti-tumor drug.

Somatostatin analogs have been also found to potentiate the cytotoxic activity of established chemotherapeutic agents in a synergistic or additive manner. The inhibitory effect of octreotide in combination with anti-cancer drugs like Mitomycin C, doxorubicin, and taxol on the growth of AR42J cancer cells, was investigated in vitro. Octreotide enhanced the anti-neoplastic effects of all the above anti-cancer drugs in a synergistic fashion (Weckbecker et al., 1996). Nagy et al. (1993) conjugated methotrexate to the somatostatin analog RC-121, with the aim of selectively targeting cytotoxic drugs to tumors, overexpressing somatostatin receptors. Such conjugates were found to be more active than RC-121 or free methotrexate in the inhibiting the growth of human pancreatic and prostate carcinoma xenografts in nude mice (Plonowski et al., 1999). Octreotide has also been found to enhance the anti-neoplastic effects of tamoxifen and ovariectomy on DMBA induced rat mammary carcinomas (Weckbecker et al., 1994). Bontenbal et al. (1998) compared the feasibility, endocrine and anti-tumor effects of a triple therapy with tamoxifen, octreotide and an anti-prolactin agent, CV205-502 as compared to tamoxifen alone. A randomized study (with long term follow up) was done in 22 post menopausal patients with metastatic breast cancer. An objective response was found in 36% of the patients treated with tamoxifen alone whereas, 55% of the patients, treated with the combination therapy, exhibited an objective response. The patients receiving triple therapy also manifested a more uniform suppression of IGF-I, GH, prolactin in the plasma. The anti-estrogen-octreotide combination also seems to have a more favorable long term toxicity profile (Ingle et al., 1999). Somatostatin analogs RC-121 and RC-160 have been used as adjuncts to LHRH agonists in the treatment of nitrosamine induced pancreatic cancer in hamsters, and R-3327H prostate cancer in rat (Weckbecker et al., 1993).

The initiation of cancer therapy with somatostatin analogs is optimal when tumors express somatostatin receptors. However, tumors change their properties during
progression. Prostate and breast cancers express receptors for androgen and estrogen respectively, in an early stage but they become hormone insensitive at an advanced stage of the disease. Similarly, somatostatin receptors seem to be useful markers of a less malignant, more differentiated stage of a tumor (Weckbecker et al., 1993). In contrast advanced tumors are qualitatively and quantitatively heterologous with respect to their cell surface receptors and antigens. All this information strongly argues for an early onset of somatostatin analog therapy when tumors are still small, homogeneously expressing somatostatin receptors, and tumor spread is absent or still at the stage of micrometastases. An advanced tumor that is heterogeneous in the expression of somatostatin receptors may be only partially responsive to somatostatin analogs.

7. MECHANISMS OF THE ANTI-NEOPLASTIC ACTION OF SOMATOSTATIN ANALOGS:
Somatostatin analogs may inhibit the growth of tumors by triggering on the signaling cascades that negatively control cell growth. These include the “direct mechanism” that are sequellae of the binding of somatostatin analogs to somatostatin receptors on neoplastic cells. The “indirect mechanisms” are related to the effects of the binding of somatostatin analogs to somatostatin receptors present on normal cells of the host (Pollak and Schally, 1998). The somatostatin receptor mediated control of tumor cell growth regulation involves multiple signaling pathways, summarized in Fig. VIII and Table 2B.

The coupling of individually expressed somatostatin receptor subtypes to G-proteins and various effectors have been extensively studied. All the five SSTR subtypes, and certainly the human isoforms are functionally coupled to the inhibition of adenyl cyclase (Table 2B) (Patel et al., 1997). Somatostatin receptors have been found to stimulate protein tyrosine phosphatase (Pollak and Schally, 1998). SSTR1 stimulates Na⁺/H⁺ exchanger via a pertussis toxin insensitive mechanism (Hou et al., 1994). SSTR2 has been found to suppress calcium channels in rat islet cells and neurons (Patel et al., 1997). SSTR4 activates phospholipase A-2 dependent arachidonate production as well as MAP kinase activity in CHO-K1 cells, both via pertussis toxin
Fig. VIII. Schematic depiction of key second messenger systems involved in somatostatin modulation of cell secretion, cell proliferation and apoptosis. (A) Induction of protein tyrosine phosphatase by somatostatin plays a key role in mediating the anti-neoplastic activity of somatostatin. (B) Mechanisms underlying the anti-secretory activity of somatostatin.

Insensitive proteins (Bito et al., 1994; Patel et al., 1997). However, SSTR3 and SSTR5 can also inhibit MAP-kinase signaling (Cordelier et al., 1997; Reardon et al., 1996). The modulation of phospholipase C/ inositol triphosphate pathway by somatostatin receptors has also been reported (Patel et al., 1997). However, only a few of these mechanisms are discussed in detail below.

7.1 DIRECT MECHANISMS OF ACTION OF SOMATOSTATIN:

7.1.1 Regulation of membrane and/or cytoplasmic phosphatases and kinases:

Regulation of tyrosine phosphorylation of cellular proteins play a major role in the control of cell growth and phosphorylation. The upregulation of intracellular protein tyrosine phosphatase (PTPase) activity triggered by binding of somatostatin analogs to SSTR2 on target cells, has received considerable attention as a direct mechanism, not only because this activity is the converse of the tyrosine kinase activity associated with many peptide mitogen receptors, but also because SSTR2 is commonly expressed on human neoplasms. The ability of somatostatin analogs like RC-160, octreotide and BIM23014 to stimulate membrane PTPase activity has been directly correlated with their inhibitory effect on cell growth. (Liebow et al., 1989). RC-160 has been found to reverse the mitogenic effect of EGF on human cancers, functionally as well as biochemically. EGF has been observed to phosphorylate proteins of 170, 65 and 60 kDa, whereas treatment of cell membranes with somatostatin analog RC-160 resulted in the dephosphorylation of these very proteins (Lee et al., 1991). Delesque et al. (1997), purified soloubilized complexes of somatostatin bound to its receptors by affinity chromatography and observed that a PTPase activity copurified with the somatostatin receptor, indicating that a tyrosine phosphatase is physically associated with the somatostatin receptor, in the cell membrane. A somatostatin sensitive 66-kDa SH-2 (Src homology 2) domain containing protein tyrosine phosphatase called PTP-1C, which dephosphorylates and inactivates growth factor receptor kinases, has been shown to translocate from the cytosol to the plasma membrane upon receptor activation to associate with somatostatin receptors (Zeggari et al., 1994; Srikant et al., 1996). The association of SSTR2 with the cytoplasmic tyrosine phosphatase has been found to be essential for the anti-proliferative activity mediated by SSTR2 (Lopez et al., 1997). However,
Reardon et al. (1997) have found that SSTR2, SSTR3 and SSTR4 also had the ability to stimulate PTPase activity in transfected NIH 3T3 cells and have suggested that SHP-2 might be implicated in the signaling pathway. Controversy exists regarding the coupling of SSTR1 to PTPase activity in cells. Data from some workers show that somatostatin induced no stimulation (Reardon et al., 1997), very low stimulation (Buscail et al., 1995), or efficient stimulation of PTPase activity (Florio et al., 1994) in CHO cells expressing SSTR1 or SSTR5. Major voids still remain in our understanding of somatostatin receptor subtype-specific molecular signals responsible for the activation of various phosphatases. Much of our current knowledge is based on CHO cells transfected with somatostatin receptor subtypes, and should be interpreted with caution, given the limitations of these systems.

The inhibition of tyrosine kinase, by somatostatin analogs, has been also implicated in the negative control of cell proliferation. The analogs octreotide, RC-160 and TT2-32 have been found to be potent inhibitors of tyrosine kinase activity (Keri et al., 1996; Pawlikowski et al., 1998). The suppression of p86 Ku autoantigen seems to be an important pathway mediating the anti-proliferative activity of somatostatin analogs like octreotide and TT2-32 (Romancer et al., 1994). The 86 kDa subunit of Ku is a somatostatin receptor playing a targeting role, in the activation of RNA polymerase by DNA dependent protein kinase and in the regulation of protein phosphatase-2A. It has been suggested that the anti-neoplastic activity of somatostatin can also be mediated by the translocation of cytosolic p86 Ku protein to the nucleus (Tovari et al., 1998).

Catteneo et al. (1996) have observed that BIM23014 inhibits cell proliferation in human neuroblastoma and small cell lung carcinoma cell lines, by the inhibition of MAP Kinase activity. This inhibition of MAP Kinase activity, is mediated via protein tyrosine phosphatase dependent inactivation of Raf-1 or guanylyl cyclase (Reardon et al., 1996; Cordelier et al., 1997; Dent et al., 1997). SSTR1 has also been shown be mediate its biological activity through the MAP kinase pathway (Florio et al., 1999). Apart from the protein tyrosine phosphatases, somatostatin has been found to activate serine threonine phosphatases (White et al., 1991) and calcium dependent phosphatase calcineurin (Renstrom et al., 1996), via pertussis toxin sensitive G-
proteins. However, the nature of the G-proteins involved and whether they couple directly or indirectly to phosphatases is unknown.

The anti-neoplastic activity of somatostatin can induce apoptosis and/or cell cycle arrest in tumor cells. Sharma et al. (1996) have shown that somatostatin induces apoptosis (and not cell cycle arrest) selectively through SSTR3. Sharma et al. (1998) have demonstrated that somatostatin signaled apoptosis in MCF-7 breast cancer cells is associated with the induction of wild type p53, Bax and acidic endonuclease. Somatostatin is also known to exert cytostatic action via other receptors like SSTR5 and induces G1 cell cycle arrest, by the induction of Rb protein (Sharma et al., 1999). Whereas apoptosis is triggered at low agonist concentration (≥ 0.1nM), cytostasis is induced at much higher (>50nM) agonist concentrations.

Studies by Todisco et al. (1994) clearly show that the inhibitory action of somatostatin may be mediated by the inhibition in the expression of early response genes. Somatostatin inhibits the expression of protooncogene c-fos and the binding of AP-1 to its regulatory DNA element in endocrine and exocrine cells (Todisco et al., 1994; Yoshotomi et al., 1997). Furthermore the inhibition of AP-1 binding and transcriptional activity is mediated via multiple classes of phosphatases.

7.1.2 Regulation of cAMP by somatostatin analogs:

The second messenger cAMP has been known to stimulate proliferation in certain mammalian cell lines, and has also been found to act as a mediator of mitogenic hormones. Furthermore, somatostatin analogs are coupled to adenylyl cyclase in intracellular signal transduction. However, somatostatin analogs do not directly affect activity of adenylyl cyclase, but they inhibit the activation of adenylyl cyclase through a possible interaction with a G protein (Carruthers et al., 1999; Qin et al., 1995). Chen et al. (1993) and Maggie et al. (1994) have also reported the inhibitory effects of somatostatin and octreotide on forskolin induced cAMP production in human neuroblastoma cell lines and in rat pituitary tumor cells. The somatostatin analog BIM23014 has been found to suppress the proliferation of rat pituitary cells by inhibiting cAMP, through the protein kinase A pathway (Tentler et al., 1997).
Major voids still remain in our understanding of somatostatin receptor subtype-selectivity for ion channel coupling and the molecular signals in the receptors responsible for the activation/inhibition of various kinases and phosphatases. The emergence of selective agonists and antagonists should greatly facilitate the study of subtype-selective effector coupling of endogenous somatostatin receptors in normal and cancer cells.

7.2 INDIRECT MECHANISMS OF ACTION OF SOMATOSTATIN:

The anti-neoplastic action of a somatostatin analog is classified as indirect if it is a consequence of binding of the analog to somatostatin receptors present on normal cells of the host. Reubi (1985) demonstrated that octreotide is a potent inhibitor of Swarm’s chondrosarcoma, an experimental neoplasm which lacks the somatostatin receptors. These chondrosarcoma cells have abundant IGF-1 receptors and it was proposed that this growth inhibition is mediated indirectly through octreotide induced suppression of pituitary GH secretion, which in turn leads to reduction in GH dependent hepatic IGF-I expression.

The GH-IGF-I axis has an important influence on the biological behavior of many neoplasms. Many types of neoplastic cells display IGF-I receptors and respond mitogenically to insulin like growth factors present in their microenvironment (Pollak and Schally, 1998). The suppressive effect of somatostatin analogs on serum IGF-I levels may be related to the direct inhibition of IGF-I gene expression as well as to the suppression of GH which in turn inhibits IGF-I. The direct suppressive action remains incompletely understood, and it is possible that the peptide suppresses autocrine and paracrine IGF-I expression as well as circulating IGF-I levels. In acromegalics, octreotide inhibits both GH secretion by pituitary tumor and leads in parallel to reduced IGF-I blood levels. Clinical trials with octreotide in breast carcinoma patients have similarly demonstrated a lowering of IGF-I levels (Ingle et al., 1999).
7.2.1 Regulation of tumor angiogenesis by somatostatin analogs:

Angiogenesis refers to the growth of new blood vessels. It is a complex multistep process consisting of the proliferation, migration of endothelial cells and finally their differentiation into capillary tube like structures (Fig. IX). Direct and indirect evidence from many studies has proved that progressive tumor growth is angiogenesis dependent (Price et al., 1997; Folkman, 1995). Neovascularization is essential for tumor cells to obtain nutrients necessary for their survival, as well as to dispose waste material. Folkman (1992) has clearly proved that a primary tumor cannot grow beyond 2mm$^3$ in the absence of neovascularization. Hence, the inhibition of tumor angiogenesis is considered as one of the most promising strategies that might lead to the development of anti-neoplastic therapies (Augustin, 1998; O'Reilly et al., 1997; Boehm et al., 1997).

The vascular endothelium is a quiescent tissue, with a very low turnover rate under physiological conditions. However, the onset of neoplasia causes a switch to the angiogenic phenotype, which permits the rapid growth of endothelial cells. The angiogenic cascade in neoplasia usually starts with a conducive microenvironment (hypoxia, acidosis) and the activation of tumor specific oncogenes which leads to the expression of endothelial cell growth factors usually referred to as “angiogenic inducers” like vascular endothelial growth factor-A (VEGF-A) (Augustin, 1998). Such angiogenic activation through specific and pleotropic growth factors induces a complex response program in endothelial cells (Fig. X). This includes the activation of autocrine growth systems; the expression and upregulation of specific cytokine and growth factor receptors; molecules regulating the proteolytic balance of cells; adhesion molecules and chemotactic factors to enhance the angiogenic cascade and finally the formation of a complex three dimensional array of blood capillaries, orchestrated by a network of cytokines and angiopoetins like bFGF, PD-ECGF, TGF etc. (Folkman, 1997).

The therapeutic ability of somatostatin and its analogs in vitro and in vivo has been suggested to depend at least in part on its effect on the development of blood vessels. The anti-angiogenic activity of octreotide has been studied in several experimental models like the CAM model, HVPAM (Human placental vein angiogenesis model),
Fig. IX. Seven critical steps of the angiogenic cascade. (a) Endothelial cells are activated by an angiogenic stimulus. (b) Secretion of proteases by endothelial cells, which causes degradation of the basement membrane an extracellular matrix. (c) A capillary sprout is formed by directed migration of endothelial cells. (d) Growth of the capillary sprout. (e) Formation of a lumen and a new basement membrane. (f) Formation of a capillary loop from two sprouts. (g) Formation of second generation capillary sprouts.

Fig. X. Sequential model of the angiogenic cascade, during tumor angiogenesis. (1) A conducive microenvironement mediates (2) expression of tumor specific endothelial cell growth factors. (3) The “angiogenic cocktail” comprising of other autocrine and paracrine growth factors. (4) Autocrine growth control of endothelial cells in the angiogenic cascade. (5) Recruitment of leukocytes and monocytes by endothelial cells and tumor-cell derived chemokines. (6) Formation and alignment of new capillaries. (a/b) FGF: acidic/basic fibroblast growth factor ; PD-ECGF: platelet-derived endothelial cell growth factor ; PIGF: placenta growth factor; TGF: transforming growth factor.

rat cornea assay, rat aorta explants grown on fibrin or fibronectin and rat mesentric windows (Danesi et al., 1996 and 1997; Woltering et al., 1997). Barrie et al. (1993) have evaluated the ability of several somatostatin analogs to inhibit angiogenesis, using the CAM model. It was found that RC-160 and octreotide were the most potent inhibitors of neovascularization. The presence of the lysine residue at position 5 and the disulfide bond between the cysteine residues at positions 2 and 7 of somatostatin analogs appears to be essential for its anti-angiogenic activity. It is conjectured that the cyclic structure of octreotide and RC-160 allows the lysine residue (at position 5) to present itself in the correct spatial orientation, which is necessary for its anti-angiogenic activity.

The anti-angiogenic activity of octreotide does not involve the protein kinase C or tyrosine phosphatase pathway. Studies by Danesi et al. (1997) suggest that octreotide inhibits paracrine angiogenic factors of the FGF family (like FGF-3), which play a critical role in the proliferation and neovascularization of endothelial cells. In addition, octreotide may exhibit its anti-angiogenic activity by inhibiting the effects of mitogenic hormones like EGF, IGF-I.

Barrie et al. (1993) determined the anti-angiogenic activity of several somatostatin analogs and found that octreotide and RC-160 were the most potent inhibitors of neovascularization. This seems to suggest that the predominant anti-angiogenic effect was displayed by somatostatin analogs with high affinity for SSTR2 (such as octreotide and RC-160). Hence, the efficiency of prevalence of SSTR2 on vascular stroma is a key factor governing the efficacy of somatostatin analogs. Reubi et al. (1994) has found that a high density of somatostatin receptors are expressed in veins surrounding human tumors. The somatostatin receptor subtype SSTR2 is preferentially expressed in these veins surrounding human cancerous tissue. These somatostatin receptors seem to play a regulatory role in hemodynamic tumor-host interactions, possibly involving angiogenesis.

The cloning of five somatostatin receptors subtypes has made it possible to characterize the structure and molecular pharmacology of this receptor family. The rich pattern of expression of somatostatin receptors throughout the brain and
peripheral tissues, coupled with the potent biological effects they elicit, clearly suggests that somatostatin receptors represent a major class of inhibitory receptors that play an important role in modulating higher brain function, secretory processes, cell proliferation and apoptosis. Synthetic analogs of somatostatin have been in clinical use for the past 10 years and now occupy an important therapeutic niche both in diagnosis and treatment of tumors. These are however only the first generation of compounds which interact with 3 of the 5 somatostatin receptors. The development of more selective agonists as well as subtype-specific antagonists, should greatly expand the scope of somatostatin pharmacotherapy. Future studies will need to define the function of individual subtypes, the role of multiple subtypes in the cell, the downstream signaling pathways responsible for the inhibition of tumor cell growth, angiogenesis and apoptosis. The molecular basis of receptor upregulation and the mechanisms underlying the differential desensitization responses in tumor cells compared to normal cells, are yet to be fully understood. Finally, a great deal needs to be learned about the biology of somatostatin receptor dysfunction in neurological, gastrointestinal and immunological disorders.