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# Structure-Function Relationships of the Signaling System for the Somatostatin Peptide Hormone Family[1]

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SYNOPSIS. **Somatostatins are a diverse family of peptide hormones that regulate a vast array of biological processes in vertebrates, including the modulation of growth, development, and metabolism. The multi-functional nature of the somatostatin family arises from the an elaborate, multi-faceted signaling system consisting of somatostatin signaling molecules, G-protein-coupled receptors, and cellular effector pathways. A striking aspect of this signaling system is the substantial diversity at every level. The signal molecules themselves display considerable structural heterogeneity. This molecular heterogeneity results from tissue-specific differential processing of a single large precursor protein (preprosomatostatin) as well as from the existence of multiple somatostatin genes, each giving rise to different precursors. In addition, numerous SS receptor subtypes have been characterized (five in mammals), some of which exhibit preferential binding to one ligand form over another. Propagation of the signal results from linkage of the receptors via numerous types of G-proteins to several different cellular effector pathways, including adenylyl cyclase, various protein kinases, numerous ion channels, and phospholipase C/inositol-3-phosphate. Ultimately, a particular response in a given target cell may be determined by structural interactions between and among the various elements of the signaling system.**

## INTRODUCTION

In the mid to late 1960s, considerable research on the brain-pituitary relationship was underway, and much of the effort was directed at identifying the factors (hypophysiotropins) that controlled the release of adenohypophysial hormones. During studies to evaluate growth hormone (GH) releasing activity, Krulich *et al.* (1968) identified both stimulatory and inhibitory effects of hypothalamic extracts on GH release from rat pituitaries *in vitro* and proposed a dual regulation model of pituitary GH release. This model was, ultimately, supported and now extends to virtually all of the other pituitary factors. The first characterization of a somatostation by Brazeau *et al.* (1973), which yielded a 14-amino acid peptide (SS-14) that inhibited GH both *in vivo* and *in vitro,* came from a search for a GH releasing factor in an extract of sheep hypothalamic fragments originally collected for the isolation of gonadotropin releasing factors.

Since the original isolation of SS-14 (Brazeau *et al.,* 1973), continued research has revealed the existence of a diverse family of peptide hormones. Today, the somatostatin (SS) family is known to display significant structural heterogeneity; numerous variants differing in both amino acid chain-length and amino acid composition have been isolated (Conlon *et al.,* 1997). Somatostatins are yet another example of factors exhibiting brain-gut distribution, being produced by neurons as well as by epithelial cells. Somatostatins have been found broadly in the central (*e.g.,* cerebral cortex, cerebellum, pineal, olfactory lobe, hypothalamus, spinal cord) and peripheral nervous systems, gastrointestinal tract (*e.g.,* salivary glands, stomach, intestine), urogenital tract (*e.g.,* bladder, prostate, collecting ducts of the kidney), pancreatic islets, adrenal glands, thyroid tissue, and placenta as well as in cerebral spinal fluid, blood,

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TABLE 1. *Physiological actions of somatostatins.*

| Class of action | Example | Reference |
|---|---|---|
| Growth | Inhibit proliferation of cultured intestinal mucosa cells | Stange *et al.,* 1984 |
| Differentiation | Inhibit differentiation of cartilage and bone cells | Weiss *et al.,* 1981 |
| Modulation of functional development | Effects on lymphocytes, monocytes, and macrophages | Chao *et al.,* 1995 |
| Secretotropic | Modulation of GH, PRL, ACTH, TSH, GLU, PP, hCG, hSL, PTH, CT, aldosterone, exocrine pancreas | Wass, 1989; Patel, 1992 |
| Neurotropic | Neurotransmitter/neuromodulator | Wass, 1989; Patel, 1992 |
| Myotropic | Modulation of vascular/visceral smooth muscle and cardiac muscle contraction | Gunshefski *et al.,* 1992 |
| Transport | Modulation of intestinal transport of $Na^+$ and $Cl^-$ | Bern *et al.,* 1985 |
| Metabolism | Stimulate breakdown of stored lipid and carbohydrate reserves | Sheridan, 1994 |

Abbreviations: GH, growth hormone; PRL, prolactin; ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating hormone; GLU, glucagon; PP, pancreatic polypeptide; hCG, human chorionic gonadotropin; hSL, human somatolactin; PTH, parathyroid hormone; CT, calcitonin.

and saliva (Reichlin, 1982; Gerich, 1983; Wass, 1989; Patel, 1992). Somatostatins are often co-localized with other factors (*e.g.,* norepinephrine, CCK, neuropeptide-Y, CGRP, GABA, VIP, substance P) within the same cell (Gibbons, 1989).

Somatostatins also possess a vast diversity of physiological actions. In addition to secretotropic effects (including the effect on GH secretion for which the family was named), somatostatins have been reported to have neurotropic and myotropic effects as wells as effects on transport, metabolism, growth, differentiation, and modulation of functional development (Table 1). It should be noted that there is overlap between and among these somewhat arbitrary classes of action. For example, the inhibition of GH secretion clearly affects growth and the inhibition of insulin secretion clearly affects metabolism. At the same time, the inhibition of GH also impacts metabolism while the inhibition of insulin also has ramifications on growth (Norman and Litwack, 1997). Similarly, behavioral effects of SSs (Rubinow *et al.,* 1987) may be explained by one or more of the actions listed in Table 1. In addition to such actions which result in physiological "cross talk," SSs also have direct effects on the various classes of action. For example, SSs have been shown to affect growth (*e.g.,* proliferation) and intermediary metabolism (*e.g.,* lipolysis) directly in target cells (Patel, 1992; Sheridan, 1994). Considering these various roles, SSs may be of considerable importance in various diseases including, neuroendocrine tumors, diabetes mellitus, epilepsy, Alzheimer and Huntington Diseases, and AIDS (Lamberts *et al.,* 1991; Patel *et al.,* 1995).

It is somewhat ironic that a hormone that was discovered by accident has presented some of the most intriguing challenges in endocrinology, among which include the elucidation of the origins of such a diverse family of peptide hormones and an understanding of the basis of the family's multifunctional nature. Comprehensive reviews of the evolution (Conlon *et al.,* 1997; Sheridan *et al.,* 1997) and the biological actions (Gerich, 1983; Epelbaum, 1986; Wass, 1989; Lamberts *et al.,* 1991; Patel, 1992; Sheridan, 1994) of the SS family have appeared previously. This review will examine elements of SS structure and its signaling systems so as to provide insight into the basis for the multiple actions of one of the most structurally and functionally diverse families of peptide hormones.

## SOMATOSTATIN STRUCTURE

### *Molecular heterogeneity*

Peptides varying in length from 14 to 37 amino acids and/or in amino acid composition, depending on species, have been iso-

lated from representatives of every extant class of vertebrates (Table 2). The molecular heterogeneity of the SS family reflects tissue-specific variations in biosynthesis from larger precursor molecules as well as the existence of multiple SS genes. The different forms of somatostatin observed in mammals (*e.g.,* SS-28) are N-terminal extensions of SS-14 and result from differential processing of the same precursor, preprosomatostatin I (PPSS I). In hypothalamus, pancreas, and stomach, the predominant pathway for the processing of PPSS I yields SS-14; whereas, in the intestine and certain other regions of the brain, SS-28 appears to be the predominant peptide form (Conlon, 1989). The isolation of alternate forms of somatostatin (*e.g.,* a 25-amino acid peptide from sheep brain; Bohlen *et al.,* 1980) suggests that alternative pathways of processing PPSS I also may exist in mammals.

The distribution of SS-14 among nonmammalian vertebrates reads, as Reichlin (1982) states, "like a passenger list on Noah's arc," and suggests that there has been strong selection to conserve the tetradecapeptide. A peptide identical in structure to that originally isolated from sheep brain (Brazeau *et al.,* 1973) has been isolated from representatives of every major group of vertebrates examined to date, from jawless fish to mammals, with the exception of the holocephalans (*e.g.,* ratfish, *Hydrologus colliei*) and the chondrosteans (*e.g.,* sturgeon, *Acipenser gueldenstaedit*) (Table 2).

Lamprey, numerous teleost fish, and frogs possess preprosomatostatins (PPSSs) in addition to PPSS I (Table 2). In teleost fish, for example, variant forms of SS that are 25- or 28-amino acids in length, depending on species, and which contain [$Tyr^7$, $Gly^{10}$]-SS-14 at their C-terminus, are known for coho salmon (*Oncorhynchus kistuch*) (Plisetskaya *et al.,* 1986), eel (*Anguilla anguilla*) (Conlon *et al.,* 1988*b*), goldfish (*Carassius auratus*) (Uesaka *et al.,* 1995), flounder (*Platichythes fleus*) and sculpin (*Cottus scorpius*) (Conlon *et al.,* 1987), and tilapia (*Oreochromis nilotica*) (Nguyen *et al.,* 1995). In addition, a unique 22-amino acid SS variant was isolated from catfish (*Ictalurus punctatus*) (Magazin *et al.,* 1982). The suggestion that the variant peptides derive from a gene different from that giving rise to PPSS I was first proposed by Hobart *et al.* (1980) based on studies of anglerfish (*Lophius americanus*) in which two distinct cDNAs were obtained from the endocrine pancreas that encoded for separate PPSSs, PPSS I (containing SS-14) and PPSS II (containing [$Tyr^7$, $Gly^{10}$]-SS-14). In rainbow trout (*O. mykiss*), we have characterized three cDNAs: one encoding PPSS I which contains SS-14 and two that encode different PPSS IIs, each containing [$Tyr^7$, $Gly^{10}$]-SS-14 (Fig. 1; Moore *et al.,* 1995, 1999; Sheridan *et al.,* 1997; Kittilson *et al.,* 1998). Goldfish also have been reported to possess three distinct cDNAs: one encoding SS-14, a second encoding [$Glu^1$, $Tyr^7$, $Gly^{10}$]-SS-14, and a third encoding [$Pro^2$]-SS-14 (Lin *et al.,* 1999). While no cDNA information exists for lampreys, recent information suggests that the multiple SSs of frogs (*Rana ridibunda*) (*e.g.,* SS-14 and [$Pro^2$, $Met^{13}$]-SS-14) arise from separate genes as well (Tostivint *et al.,* 1996).

*Functional differences of isoforms*

The physiological significance of the variant SS forms is indicated by several lines of evidence. First, numerous reports indicate that the variant forms of SS are secreted; this is the case for both mammals (*e.g.,* SS-14 and SS-28) (Patel, 1992) and teleost fish (*e.g.,* SS-14 and salmonid SS-25 with [$Tyr^7$, $Gly^{10}$]-SS-14 at its C-terminus) (Eilertson and Sheridan, 1995). Second, that the secretion profile, especially that of mammals, is not merely a consequence of the biosynthetic pathway is suggested by ample evidence of tissue specific processing (Patel and O'Neil, 1988). Third, the secretion of SS-14 and of variants containing [$Tyr^7$, $Gly^{10}$]-SS-14 is differentially controlled (Eilertson and Sheridan, 1995; Eilertson *et al.,* 1995, 1996). Moreover, the two PPSS IIs of rainbow trout (PPSS II′ and PPSS II″) that encode [$Tyr^7$, $Gly^{10}$]-SS-14 at their C-terminus are differentially expressed (Moore *et al.,* 1999).

As the variant forms of native somatostatins were discovered, it became increasingly clear that there were important struc-

TABLE 2. *A comparison of amino acid sequences of somatostatin peptides.*[a]

| | −23 −20 −10 +1 +10 +14 |
|---|---|
| HAGFISH SS-14 (I)[b]○ | A G C K N F F W K T F T S C |
| HAGFISH 22-34 (I)[b]○ | A V E R P R Q D G Q V H E P P G R E R K A G C K N F F W K T F T S C |
| LAMPREY | |
| PETROMYZON | |
| SS-14 (I)[c]○ | A G C K N F F W K T F T S C |
| SS-14 (II)[d]○ | A G C K N F F W K T F S S C |
| SS-34 (II)[d]○ | A A A V A G S P Q Q L L P L G Q R E R K A G C K N F F W K T F S S C |
| SS-37 (II)[d]○ | A L R A A A V A G S P Q Q L L P L G Q R E R K A G C K N F F W K T F S S C |
| LAMPETRA | |
| SS-14 (I)[e]○ | A G C K N F F W K T F T S C |
| SS-35 (II)[e]○ | A A A A P G A A G G A Q L P L G N R E R K A G C K N F F W K T F S S C |
| GEOTRIA | |
| SS-33 (II)[f]○ | A V Q E A G G A A M P P P G Q R D R K A G C K N F F W K T F S S C |
| TORPEDO SS-14 (I)[g]○ | A G C K N F F W K T F T S C |
| RATFISH SS-14 (I?)[h]○ | A G C K S F F W K T F T S C |
| BOWFIN SS-14 (I?)[i]○ | A G C K N F F W K T F T S C |
| BOWFIN SS-26 (I?)[i]○ | S A N * * P A L A P R E R K A G C K N F F W K T F T S C |
| STURGEON SS-14 (I?)[j]○ | A P C K N F F W K T F T S C |
| CATFISH SS-14 (I)[k]○ | A G C K N F F W K T F T S C |
| CATFISH SS-22 (II?)[l]○ | D N T V R S K P * * * * * L N C M N Y F W K S S T A C |
| EEL SS-14 (I)[m]○ | A G C K N F F W K T F T S C |
| EEL SS-25 (II)[m]○ | S V D * * * N Q Q G R E R K A G C K N F Y W K G P T S C |
| SALMON SS-14 (I)[n]○ | A G C K N F F W K T F T S C |
| SALMON SS-14 (II)[n]○ | A G C K N F Y W K G F T S C |
| SALMON SS-25 (II)[n]○ | S V D * * * N L P P R E R K A G C K N F Y W K G F T S C |
| TROUT SS-26 (I)○■ | A P * * G P V L A P R E R K A G C K N F F W K T F T S C |
| TROUT SS-28 (II)[p]■ | S V G N P N N L P P R E R K A G C K N F Y W K G F T S C |
| TROUT SS-25 (II)[q]■ | S V D * * * N L P P R E R K A G C K N F Y W K G F T S C |
| ANGLERFISH SS-28 (I)[r&s]■ | A A S G G P L L A P R E R K A G C K N F F W K T F T S C |
| ANGLERFISH SS-28 (II)[r&s]■ | S V D S T N N L P P R E R K A G C K N F Y W K G F T S C |
| GOLDFISH SS-14 (I)[t]■ | A G C K N F F W K T F T S C |
| GOLDFISH SS-14 (???)[t]■ | A P C K N F F W K T F T S C |
| GOLDFISH SS-28 (II)[t]■ | S A E S S N Q L P T R V R K E G C K N F Y W K G F T S C |
| GOLDFISH SS-28 (II)[u]○ | S V E S S N H L P A R E R K A G C K N F Y W K G F T S C |
| FLOUNDER SS-14 (I)[v]○ | A G C K N F F W K T F T S C |
| FLOUNDER SS-28 (II)[v]○ | S I E P P N N L P P R E R K A G C K N F Y W K G F T S C |
| SCULPIN SS-14 (I)[v]○ | A G C K N F F W K T F T S C |
| SCULPIN SS-28 (II)[v]○ | S V D P P N N I P L R E R K A G C K N F Y W K G F T S C |
| TILAPIA SS-14 (I)[w]○ | A G C K N F F T K T F T S C |
| TILAPIA SS-28 (II)[w]○ | S A D Q P N S I P P R E R K A G C K N F Y W K G L T S C |
| FROG SS-14 (I)[x&Y]■ | A G C K N F F W K T F T S C |
| FROG SS-14 (II)[x&y]■ | A P C K N F F W K T F T M C |
| SALAMANDER SS-14 (I)[z]○ | A G C K N F F W K T F T S C |
| ALLIGATOR SS-14 (I)[aa]○ | A G C K N F F W K T F T S C |
| PIGEON SS-14 (I)[bb]○ | A G C K N F F W K T F T S C |
| CHICKEN SS-14 (I)[cc]■ | A G C K N F F W K T F T S C |
| OVINE SS-14 (I)[dd]○ | A G C K N F F W K T F T S C |
| OVINE SS-25 (I)[ee]○ | S N P A M A P R E R K A G C K N F F W K T F T S C |
| OVINE SS-28 (I)[ee]○ | S A N S N P A M A P R E R K A G C K N F F W K T F T S C |
| BOVINE SS-14 (I)[ff]■ | A G C K N F F W K T F T S C |
| RAT SS-14 (I)[gg&hh]■ | A G C K N F F W K T F T S C |
| MONKEY SS-14 (I)[ii]■ | A G C K N F F W K T F T S C |
| HUMAN SS-14 (I)[jj]■ | A G C K N F F W K T F T S C |

[a] Sequences are presented using single amino acid codes and are arranged for maximum alignment (* denotes deletion). ○ sequences derived directly from amino acid analysis. ■ sequences predicted from cDNA. Peptides presumed to be derived from preprosomatostin I are designated I; peptides presumed to be derived from preprosomatostatin II are designated II (underlined residues indicate those characteristic of preprosomatostatin (II).

References: [b] Conlon *et al.,* 1988a; [c] Sower *et al.,* 1994; [d] Andrews *et al.,* 1988; [e] Conlon *et al.,* 1995a; [f] Conlon *et al.,* 1995b; [g] Conlon *et al.,* 1985; [h] Conlon, 1990; [i] Wang *et al.,* 1993; [j] Nishi *et al.,* 1995; [k] Andrews and Dixon, 1981; [l] Oyama *et al.,* 1980; [m] Conlon *et al.,* 1988b; [n] Plisetskaya *et al.,* 1986; [o] Kittilson *et al.,* 1998; [p] Moore *et al.,* 1995; [q] Sheridan *et al.,* 1997; [r] Hobart *et al.,* 1980; [s] Goodman *et al.,* 1980; [t] Lin *et al.,* 1999; [u] Uesaka *et al.,* 1995; [v] Conlon *et al.,* 1987; [w] Nguyen *et al.,* 1995; [x] Vaudry *et al.,* 1992; [y] Tostivint *et al.,* 1996; [z] Cavanaugh *et al.,* 1996; [aa] Wang and Conlin, 1993; [bb] Spiess *et al.,* 1979; [cc] Nata, 1991; [dd] Brazeau *et al.,* 1973; [ee] Bohlen *et al.,* 1980; [ff] Su *et al.,* 1988; [gg] Goodman *et al.,* 1983; [hh] Funckes *et al.,* 1983; [ii] Travis and Sutcliffe, 1988; [jj] Shen *et al.,* 1982.

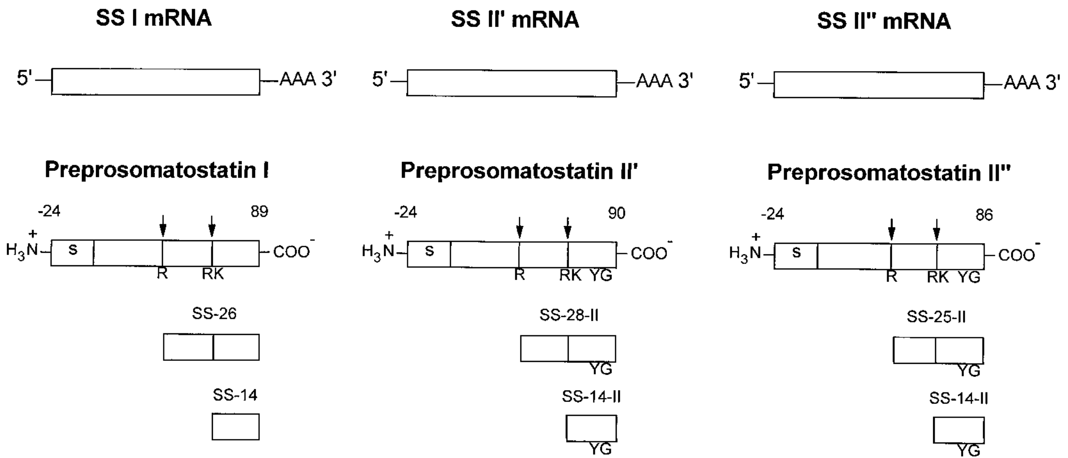


FIG. 1. Schematic representation of the biosynthesis of somatostatins from multiple somatostatin genes in rainbow trout. The arrows indicate putative cleavage sites.

ture-function relationships. One of the first actions evaluated was that of growth hormone inhibition. In mammals, SS-28 appeared more potent than SS-14 in inhibiting GH release from rat pituitary fragments *in vitro* (Brazeau *et al.,* 1981). In teleost fish, SS-14 and SS-28 appeared equipotent in their ability to inhibit GH release from goldfish pituitary fragments (Marchant *et al.,* 1987). By contrast, neither catfish SS-22, a unique variant of SS, nor salmonid SS-25, which contains [Tyr$^7$, Gly$^{10}$]-SS-14 at its C-terminus, had any effect on GH release from goldfish pituitary fragments (Marchant *et al.,* 1987; Marchant and Peter, 1989), suggesting that these molecular forms of SS do not possess the biological activity for which they are named.

Structure-function relations also have been observed in SS modulation of endocrine pancreas function. In mammals, *in vivo* administration of SS-14 appeared to reduce insulin and glucagon levels to the same extent (Koerker *et al.,* 1974); however, *in vitro* experiments suggest that SS-14 is a more potent inhibitor of insulin than of glucagon (Gerich *et al.,* 1975). In addition, while SS-25 and SS-28 were more potent than SS-14 in inhibiting insulin release from *in vitro* perfused rat pancreas, SS-14 preferentially inhibited glucagon release (Mandarino *et al.,* 1981). In teleost fish, injection of salmonid SS-25 but not SS-14 reduced plasma insulin levels in rainbow trout (Fig. 2; Eilertson and Sheridan, 1993). In addition, salmonid SS-25 appeared more potent than SS-14 in reducing plasma glucagon levels in rainbow trout (Eilertson and Sheridan, 1993).

*Analogs*

The wide-ranging biological effects of the SS family has spawned intense pharmaceutical interest to develop compounds with selective actions for clinical use. Many analogs of SS have been synthesized in an effort to evaluate critical structural elements that are important in understanding activity, potency, and bioavailability. Initial substitution-replacement studies that relied upon the inhibition of GH from rat pituitary fragments as a bioassay revealed several important structural features (Vale *et al.,* 1975). First, the amino terminus of SS-14 does not appear to be required for biological activity. Second, the disulfide bond appears important for bioactivity, but the size of the ring does not appear to be crucial. Subsequent studies have indicated that the sequence Phe-Trp-Lys-Thr, corresponding to positions 7–10 and which constitute a β-turn, is the critical region of native SSs (Raynor and Reisine, 1992).

Various classes of analogs have been made (Reisine and Bell, 1995*a*), including amino acid-deleted or -substituted SS-14 compounds, dicarba analogs, bicyclic octapeptide analogs (*e.g.,* SMS201-995, some-

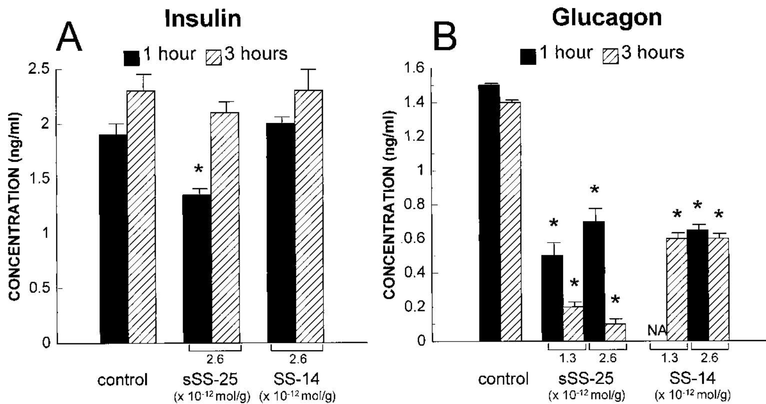


FIG. 2. Differential effects of SS-14 and salmonid SS-25 on the plasma concentration of (A) insulin and (B) glucagon in rainbow trout. Data presented as means ± SEM (n = 8–10); *significantly different from respective control. (Redrawn from Eilertson and Sheridan, 1993)

times known as octreotide or Sandostatin®), and cyclic hexapeptides (*e.g.,* MK687). Such analogs are more potent and longer acting than native SS-14. For example, the half-life of SS-14 has been estimated to be 1–3 min in humans (Sheppard *et al.,* 1979); whereas, the half-life of SMS201-995 is about 80 min and has been useful for the treatment of various diseases including acromegaly (Wass, 1989). The use of SS analogs is necessary for studying somatostatin receptor binding because the native forms of SS-14 and SS-28 from PPSS I lack Tyr residues that can be iodinated to make $^{125}$I-labeled ligands; however, this is not the case for teleost PPSS II which contains [Tyr$^7$, Gly$^{10}$]-SS-14 at its C-terminus.

## SOMATOSTATIN RECEPTORS

### *Distribution and binding characteristics*

The actions of SSs are initiated by specific binding to receptors on target cells. Somatostatin specific binding was first described in a rat somatotroph cell line, $GH_4C_1$, by Schonbrunn and Tashjian (1978). Somatostatin binding was subsequently characterized in a variety of tissues and normal cells, including brain, retina, pituitary, adrenal cortex, gastrointestinal tract, pancreas, thyroid, liver, adipocytes, and lymphocytes, and cell lines, such as the pituitary cell lines $GH_3$/$GH_4C_1$ and AtT-20, hamster insulinoma, rat islet RINm5f tumor cells, AR42J and Mia $PaCa_2$ pancreatic tumor cells, and human myeloma and leukemic cell lines (Simon *et al.,* 1988; Raynor and Reisine, 1992; Patel *et al.,* 1995; Pesek and Sheridan, 1996).

Somatostatin receptors have received a great deal of attention and have been studied by binding analysis using membrane fractions and whole cells, *in vivo* and *in vitro* autoradiography, covalent crosslinking, purification of solubilized receptor protein, and molecular cloning (Rens-Domiano and Reisine, 1992; Raynor and Reisine, 1992; Reisine and Bell, 1995*a, b*; Patel *et al.,* 1995, 1996). Binding analysis has yielded a variety of information. In some cases, a single class of binding sites has been reported (Srikant and Patel, 1981*a*; Sullivan and Schonbrunn, 1987), while in other cases (even in the same tissue, but with different preparations, *i.e.,* cells *vs.* membranes), two classes of binding sites were suggested (Leitner *et al.,* 1979; Mehler *et al.,* 1980). Differences in the potency of ligands to displace [$^{125}$I]-Tyr$^{11}$-SS-14 also have been reported. For example, SS-28 was more potent than SS-14 in displacing label in mammalian pituitary preparations, whereas the opposite pattern was observed in brain (Srikant and Patel, 1981*b*). By using analogs of SS, Raynor and Reisine (1989) identified two pharmacologically distinct populations of SS in rat brain. In rainbow trout there appeared to be two distinct hepatic SS binding sites (Fig. 3). The

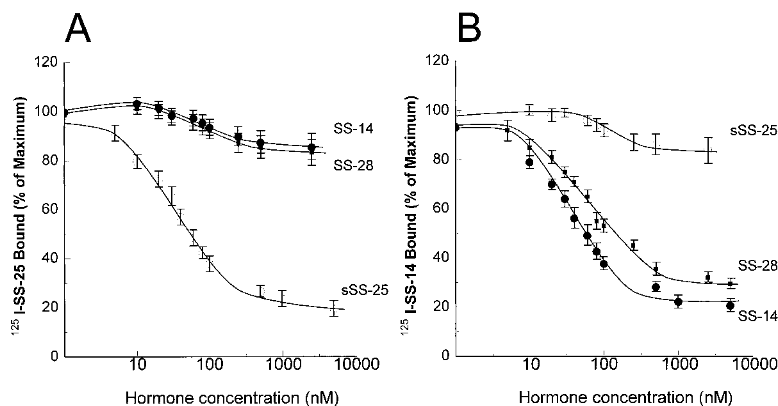


FIG. 3. Differential specificity of $^{125}$I-SS-14 and $^{125}$I-sSS-25 binding to liver membranes of rainbow trout. Membrane preparation (150 μg protein) were incubated for 16 hr at 4°C, pH 7.5. Data presented as means ± SEM (n = 3–5). sSS, salmonid somatostatin-25. ($^{125}$I-SS-14 binding data are redrawn from Pesek and Sheridan, 1996)

first was specific for PPSS I products; SS-14 was only slightly more potent than SS-28 in displacing label, whereas sSS-25 was a poor competitor with [$^{125}$I]-SS-14 (Pesek and Sheridan, 1996). The second site was specific for PPSS II products; both SS-14 and SS-28 displaced labeled [$^{125}$I]-sSS-25 only at pharmacological concentrations (Fig. 3). The use of radiolabeled ligands coupled with autoradiography has shown different patterns of labeling in brain and pancreas (Raynor and Reisine, 1992). Moreover, crosslinking experiments identified numerous proteins of varying size (21–228 kDa) that could be labeled with different $^{125}$I-labeled analogs of SS (Reisine and Bell, 1995*a*). These data along with the aforementioned information on functional differences among the native isoforms of SS laid the groundwork for the notion that multiple SS receptors exist.

*Molecular heterogeneity*

Continued research on SS receptors not only confirmed that there were SS-14 and SS-28 selective binding sites in mammals, but indicated that several subclasses of SS receptors existed. Studies that employed photoaffinity labeling coupled with purification suggested the presence of at least three species of SS receptors expressed in a tissue-specific manner and which have a molecular weight of 60–90 kDa (Patel *et al.,* 1995). Characterization of the isolated receptors indicated that they were glycoproteins; variation in the degree of glycosylation may contribute to the physical and functional variations of the subtypes (Raynor and Reisine, 1992).

Molecular cloning experiments confirmed the existence of multiple SS receptor subtypes and revealed the diverse nature of this receptor family. To date, five distinct SS receptor subtypes, designated $sst_1$–$sst_5$ (Hoyer *et al.,* 1995), have been identified. The initial approach to cloning the SS receptors was a PCR-based strategy using degenerate primers corresponding to conserved sequences in G protein-coupled receptors followed by screening of genomic libraries. The first two receptors that were characterized in this manner were human and mouse $sst_1$ and $sst_2$ (Yamada *et al.,* 1992*a*). Since this time, a variety of approaches have been used to elucidate the structures of human $sst_1$–$sst_5$, rat $sst_1$–$sst_5$, mouse $sst_1$–$sst_5$, and bovine and porcine $sst_2$ (Fig. 4). While the genes encoding ssts lack introns, at least in their coding regions, the $sst_2$ gene of human, rat, and mouse possesses a cryptic intron and two variant gene products arise through differential splicing: a long form, $sst_{2A}$, and a short form, $sst_{2B}$ (Patel *et al.,* 1996).

The SS receptor family belongs to a subgroup of receptors having seven transmembrane domains and that are coupled to G-proteins. The subgroup is distinguished by

| | rat sst1 | mouse sst1 | human sst2A | human sst2B | rat sst2 | mouse sst2A | mouse sst2B | cow sst2 | pig sst2 | human sst3 | rat sst3 | mouse sst3 | human sst4 | rat sst4 | mouse sst4 | human sst5 | rat sst5 | mouse sst5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| human sst1[a] | 98 | 92 | 44 | 45 | 50 | 50 | 47 | 46 | 47 | 37 | 38 | 36 | 63 | 62 | 61 | 49 | 47 | 48 |
| rat sst1[b] | | 92 | 44 | 48 | 50 | 50 | 47 | 46 | 47 | 38 | 39 | 36 | 62 | 62 | 61 | 48 | 47 | 48 |
| mouse sst1[a] | | | 44 | 45 | 50 | 50 | 47 | 46 | 47 | 37 | 39 | 36 | 62 | 61 | 61 | 49 | 47 | 48 |
| human sst2A[a] | | | | 93 | 94 | 96 | 87 | 95 | 97 | 42 | 43 | 45 | 46 | 47 | 47 | 56 | 57 | 57 |
| human sst2B[c] | | | | | 88 | 90 | 90 | 87 | 89 | 43 | 42 | 41 | 49 | 49 | 49 | 57 | 58 | 58 |
| rat sst2[d] | | | | | | 99 | 90 | 95 | 95 | 46 | 44 | 44 | 50 | 50 | 50 | 60 | 60 | 60 |
| mouse sst2A[a] | | | | | | | 92 | 95 | 94 | 46 | 44 | 45 | 50 | 50 | 50 | 60 | 61 | 60 |
| mouse sst2B[e] | | | | | | | | 87 | 88 | 44 | 43 | 42 | 47 | 48 | 48 | 57 | 58 | 58 |
| cow sst2[f] | | | | | | | | | 97 | 45 | 44 | 43 | 50 | 50 | 50 | 62 | 60 | 60 |
| pig sst2[g] | | | | | | | | | | 45 | 44 | 43 | 50 | 50 | 50 | 60 | 60 | 60 |
| human sst3[h] | | | | | | | | | | | 85 | 84 | 41 | 40 | 40 | 52 | 52 | 51 |
| rat sst3[i] | | | | | | | | | | | | 97 | 37 | 37 | 37 | 49 | 51 | 51 |
| mouse sst3[j] | | | | | | | | | | | | | 37 | 36 | 36 | 48 | 50 | 50 |
| human sst4[k] | | | | | | | | | | | | | | 89 | 89 | 53 | 53 | 53 |
| rat sst4[l] | | | | | | | | | | | | | | | 97 | 54 | 53 | 52 |
| mouse sst4[m] | | | | | | | | | | | | | | | | 53 | 52 | 52 |
| human sst5[n] | | | | | | | | | | | | | | | | | 84 | 84 |
| rat sst5[o] | | | | | | | | | | | | | | | | | | 97 |
| mouse sst5[p] | | | | | | | | | | | | | | | | | | |

FIG. 4. Amino acid sequence identities between members of the somatostatin receptor family. Sequences were aligned and analyzed with ClustalW. References: [a]Yamada *et al.,* 1992*a*; [b]Meyerhof *et al.,* 1991; [c]Patel *et al.,* 1993; [d]Kluxen *et al.,* 1992; [e]Vanetti *et al.,* 1992; [f]Xin *et al.,* 1992; [g]Matsumoto *et al.,* 1994; [h]Yamada *et al.,* 1992b; [i]Meyerhof *et al.,* 1992; [j]Yasuda *et al.,* 1992; [k]Xu *et al.,* 1993; [l]Bito *et al.,* 1994; [m]Schwabe *et al.,* 1996; [n]Yamada *et al.,* 1993; [o]O'Carroll *et al.,* 1992; [p]Lublin *et al.,* 1997.

the presence of the amino acid sequence Asp-Arg-Tyr (DRY) or a similar motif at the boundary of the third transmembrane domain and the second intracellular loop. The SS receptor family is most closely related to the opioid receptor family, with the two families sharing approximately 30% sequence identity (Yasuda *et al.,* 1993). The SS receptors range in size from 346 to 428 amino acids and there is 36–63% amino acid identity between the subtypes (Fig. 4). The sequences of the seven transmembrane domains are most similar, whereas the N- and C-termini are the most divergent regions. Within a subtype, the $sst_1$ sequence is the most highly conserved (94%) while the $sst_5$ sequence is the least conserved (88%). The amino acid identities for the other subtypes are 92% for $sst_2$, 89% for $sst_3$, and 92% for $sst_4$ (Fig. 4).

Each of the cloned subtypes has been expressed in heterologous cell lines (*e.g.,* CHO or COS-1) to determine the ligand binding properties and to evaluate the signal transduction system(s) through which the receptors interact. Binding studies revealed that there is selective binding of SS and their analogs to specific receptor subtypes. Native SSs, SS-14, SS-25, and SS-28, have high affinities for each of the receptor subtypes; however, $sst_5$ appears to have some preferential affinity for SS-28 (Reisine and Bell, 1995*a*). Pronounced selective binding by receptor subtypes for short analogs of SS has been observed. For example, $sst_2$ and $sst_5$ bind to SMS 201-995 with high affinity, but the affinites of $sst_1$, $sst_3$, and $sst_4$ for this ligand are low to nonexistent (Reisine and Bell, 1995*a*). Based on such pharmacological characteristics, the

TABLE 3. *Tissue distribution of somatostatin receptor subtypes.*

| Receptor subtype | Species | Tissues in which somatostatin subtypes are found |
|---|---|---|
| $sst_1$ | human[a, c] | Brain, small intestine, stomach, lung, kidney, pancreas, colon |
| | rat[e,g,i] | Brain, pituitary, pancreatic islets, stomach, small intestine, heart, spleen, adrenals, testis |
| | mouse[c] | Brain |
| $sst_2$ | human[a,c] | Brain, kidney, small intestine, colon, liver, pancreas |
| | rat[e,g,h] | Brain, pituitary, adrenals, pancreas, stomach, kidney, spleen, colon, testis |
| | mouse[l] | Brain |
| $sst_3$ | human[a,d] | Brain, pituitary, pancreatic islets |
| | rat[e,g,i] | Brain, pituitary, pancreatic islets, stomach, small intestine, liver, kidney, heart, spleen, adrenals, testis, lung |
| | mouse[m] | Brain, pancreas |
| $sst_4$ | human[a] | Brain, pituitary, stomach, lung, kidney, adrenals |
| | rat[e,i] | Brain, pituitary, pancreatic islets, stomach, small intestine, lung, kidney, heart, spleen |
| | mouse[k,n] | Brain, lung |
| $sst_5$ | human[b] | Brain, pituitary, small intestine, heart, adrenals |
| | rat[e,f,i] | Brain, pituitary, pancreatic islets, small intestine, spleen, kidney, stomach |
| | mouse[j] | Brain, pituitary, kidney, spleen, ovary, stomach, intestine, thymus |

References: [a] Rohrer *et al.,* 1993; [b] O'Carroll *et al.,* 1994; [c] Yamada *et al.,* 1992a; [d] Yamada *et al.,* 1992b; [e] Bruno *et al.,* 1993; [f] Raulf *et al.,* 1994; [g] Zhu *et al.,* 1998; [h] Patel *et al.,* 1993; [i] Patel *et al.,* 1995; [j] Baumeister *et al.,* 1998; [k] Schwabe *et al.,* 1996; [l] Vanetti *et al.,* 1992; [m] Yasuda *et al.,* 1992; [n] Bruns *et al.,* 1996.

somatostatin receptor family can be divided into two subgroups: the $sst_2/sst_5$ subgroup and the $sst_1/sst_3/sst_4$ subgroup (Bruns *et al.,* 1996).

Fundamental to understanding the role of SS receptor subtypes in the biological action of SSs is the distribution of the various subtypes among the tissues of an organism. The tissue distribution of the SS receptor subtypes has been studied by a variety of methods, including northern blotting, RNase protection assay, RT-PCR, and *in situ* hybridization. The mRNAs encoding the various receptor subtypes exhibit distinct but somewhat overlapping patterns of expression (Table 3). In humans and rats, all subtypes are expressed in the central nervous system, but to varying extents in different regions (Reisine and Bell, 1995*a*; Patel *et al.,* 1995). The distribution of subtype mRNAs in the periphery is tissue-specific. For example, the rank order of expression in the pituitary is $sst_2 > sst_1 = sst_3 > sst_5 > sst_4$ while that in the spleen is $sst_3 > sst_1 = sst_4 = sst_5 > sst_2$ (Reisine and Bell, 1995*b*). The patterns of expression of the various subtypes within particular tissues also vary with species (Reisine and Bell, 1995b; Patel *et al.,* 1996).

*Functional domains*

Selective binding of SS and its analogs to SS receptor subtypes suggests that certain structural motifs of the receptor are important for binding and initiation of biological activity. Kaupmann *et al.* (1995) used site-directed mutagenesis to identify the specific amino acids in $sst_2$ that mediate its specific interaction with the short-chain analog of SS, SMS 201-955. Specifically, these workers attempted to shift the low affinity of $sst_1$ for SMS 201-995 to high affinity binding similar to that displayed by wild type $sst_2$. A single $Ser^{305}$ to Phe mutation in transmembrane domain 7 increased the affinity of $sst_1$ for SMS 201-955 nearly 100-fold; when this mutation was combined with the additional substitution of $Gln^{291}$ to Asn in transmembrane domain 6, virtually full $sst_2$-like binding of SMS 201-955 was obtained.

These findings contribute to a model (Kaupmann *et al.,* 1995) showing that the core residues of SS-14, $Phe^6$-Phe-Trp-Lys-Thr-$Phe^{11}$, interact with a ligand binding pocket located in transmembrane domains (TMD) 3 to 7, which is lined with hydrophobic and charged amino acids. In $sst_1$, the ligand binding pocket is lined by $Phe^{287}$ and $Gln^{291}$ in TMD 6 and by $Ser^{305}$ in TMD 7

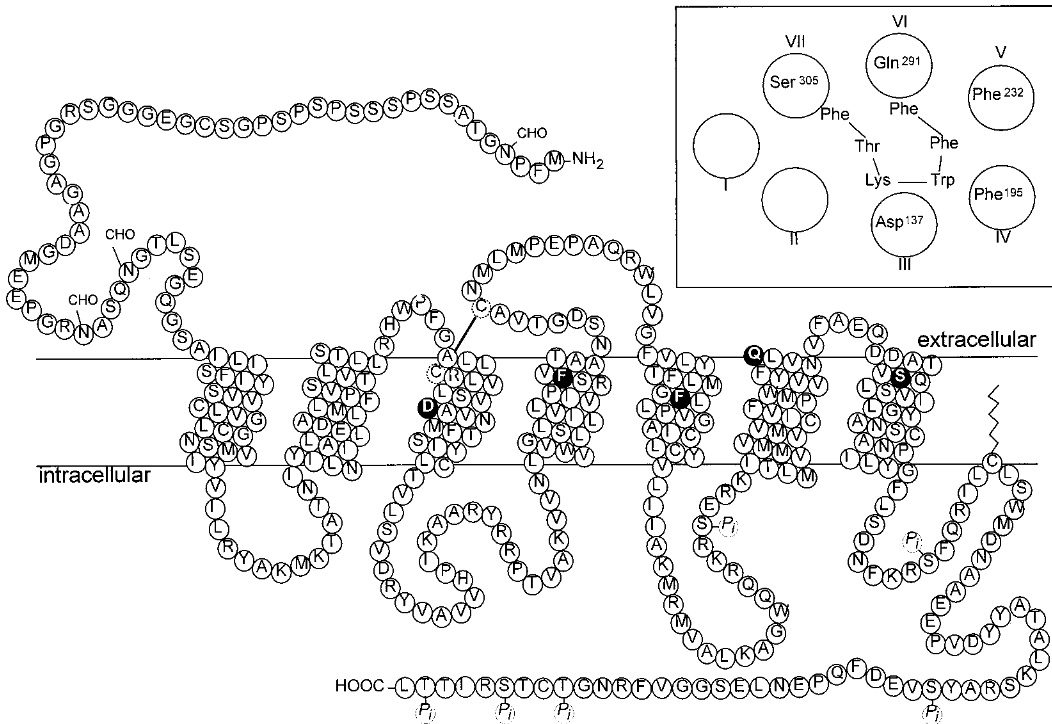


FIG. 5. Schematic representation of a somatostatin receptor in the plasma membrane of a cell. The sequence of the human subtype 1 receptor is shown. The inset shows a top view of the receptor complex and the putative binding pocket involving transmembrane domains III–VII as well as the $Phe^{6}$ to $Phe^{11}$ sequence of somatostatin-14. Filled circles denote amino acids believed to be important for ligand interaction (position numbers of these residues are noted in the inset); C−C, disulfide bond; CHO and $P_i$, potential glycosylation and phosphorylation sites, respectively; ⪋, site for a palmitoyl anchor.

(Fig. 5). This pocket provides a hydrophobic environment for interaction with the $Phe^{6}$-$Phe^{11}$ region of SS-14; this interaction may be enhanced by an electrostatic interaction between $Lys^{9}$ of the ligand and $Asp^{137}$ of TMD 3. The analog SMS 201-955 binds poorly to $sst_1$ because of the presence of specific residues ($Gln^{291}$ in TMD 6 and $Ser^{305}$ in TMD 7) which interfere with the disulfide bridge of the analog and prevent it from entering more deeply into the pocket to interact with $Phe^{287}$. The native ligand, SS-14, is larger and more flexible and can adopt a conformation suitable for entry into the pocket. Other elements of the receptor help confer binding specificity. For example, extracellular loop 2 appears to be important for receptor selectivity of agonists, including the selectivity of $sst_5$ for SS-28 (Liapakas *et al.,* 1996; Greenwood *et al.,* 1997). The nature and extent of receptor glycosylation also may influence ligand binding (Rens-Domiano and Reisine, 1991).

Equally important for SS action is the propagation of signal through the interaction of the receptor with cell effector system(s). Common to each of the various effector systems (discussed below) that transduce SS binding is the post-receptor interaction with a heterotrimeric G-protein complex. Structural features of the various SS receptors are responsible for this linkage. A putative consensus sequence for G-protein coupling appears on cytoplasmic loop 3 of each of the receptor subtypes except $sst_1$ (Patel *et al.,* 1995).

## EFFECTOR SYSTEMS

The action of SS proceeds from the binding of the ligand to the receptor and the subsequent activation of one of numerous

effector pathways. As mentioned previously, receptor activation of an effector pathway is mediated by G-proteins. The nature and extent of the interaction between a somatostatin receptor subtype and a particular G-protein element may help dictate which effector pathway is used and, ultimately, determine the specific response of the target cell.

*Multiple effector pathways*

A role of adenylyl cyclase as a mechanism of action for SS was suggested by several lines of evidence. Early studies with rat anterior pituitary cells showed that somatostatin inhibited Br8-cAMP-stimulated GH release as well as prostaglandin-induced increases in endogenous levels of cAMP (Vale *et al.,* 1975). Additional research indicated that the effect of SS on cAMP accumulation was directly on adenylyl cyclase and not an indirect action, such as via phosphodiesterase (Dorflinger and Schonbrunn, 1983). Subsequent work revealed that SS operates through numerous effector systems in addition to adenylyl cyclase, including protein phosphatases, cyclic GMP-dependent kinases, phospholipase C, $Ca^{++}$ and $K^+$ channels, and a $Na^+/H^+$ exchanger (Rens-Domiano and Reisine, 1992; Reisine and Bell, 1995*a, b*; Patel *et al.,* 1995, 1996).

Studies of cloned SS receptor subtypes transfected into assorted cell lines has been a source of much of the information about the linkage between receptors and effector systems. At times this information is not consistent, perhaps as a result of the nature of the transfection and/or the choice of cell line (Patel *et al.,* 1995). In stably transfected CHO-K1 and COS-7 cells, all five of the human sst subtypes appear to by coupled to adenylyl cyclase (Hoyer *et al.,* 1994). In addition, $sst_1$ appears to be linked via pertussis toxin-insensitive mechanisms to a protein tyrosine phosphatase and a $Na^+/H^+$ exchanger (Buscail *et al.,* 1994; Florio *et al.,* 1994; Hou *et al.,* 1994). Somatostatin receptor subtype 2 has been shown to stimulate protein tyrosine phosphatase and phospholipase C, by both pertussis toxin-sensitive and -insensitive mechanisms (Florio *et al.,* 1994; Tomura *et al.,* 1994; Buscail *et al.,* 1995). In RIN 5F cells, however, $sst_2$ is linked to the inhibition of $Ca^{++}$ influx via voltage-dependent $Ca^{++}$ channels (Fujii *et al.,* 1994). Somatostatin receptor subtype 4 ($sst_4$) activates phospholipase $A_2$ and a MAP kinase, but has no effect on phospholipase C/$IP_3$ (Buscail *et al.,* 1994). Somatostatin receptor subtype 5 inhibits CCK-stimulated $Ca^{++}$ mobilization and may have effects opposite to those of $sst_2$ on phospholipase C/$IP_3$ (Buscail *et al.,* 1994).

*Coupling to G-proteins*

The involvement of G-proteins in the signal transduction pathway of SS was initially demonstrated by observations that guanine nucleotides regulated SS-14 binding (Enjalbert *et al.,* 1983) and that GTP was required for SS-14 inhibition of adenylyl cyclase activity (Koch and Schonbrunn, 1984). Since this time, it has been discovered that there are numerous types of G-proteins. Knowledge of SS receptor/G-protein/effector system coupling is just emerging.

Some of the specific G-proteins that associate with ssts have been elucidated by immunoprecipitation approaches. Three G-protein α subunits, $G_{i\alpha 2}$, $G_{i\alpha 3}$ and $G_{o\alpha 2}$, can associate with $sst_2$ (Brown and Schonbrunn, 1993; Law *et al.,* 1993; Luthin *et al.,* 1993). Similar approaches have indicated that $sst_2$ associates with $G_{\beta 35}$ but not $G_{\beta 36}$ (Rens-Domiano and Reisine, 1992). The interactions between G-proteins and other ssts are not known.

A number of reports have appeared that suggest that there is selectivity in the G-protein coupling of SS receptors to cellular effector systems. For example, $G_{i\alpha 1}$ and $G_{i\alpha 2}$ appear to mediate sst coupling to adenylyl cyclase activity in CHO and AtT-20 cells, while $G_{i\alpha 3}$ seems to be involved in this linkage in $GH_4C_1$ cells (Law *et al.,* 1993; Luthin *et al.,* 1993; Tallent and Reisine, 1992; Senogles, 1994). In addition, the coupling of ssts to voltage-gated $Ca^{++}$ channels appears to be mediated by $G_{o\alpha 2}$ and that to $K^+$ channels is mediated by $G_{i\alpha 3}$ (Yatani *et al.,* 1987; Taussig *et al.,* 1992).

## CONCLUSIONS

Somatostatins are multi-functional. They coordinate a vast array of physiological

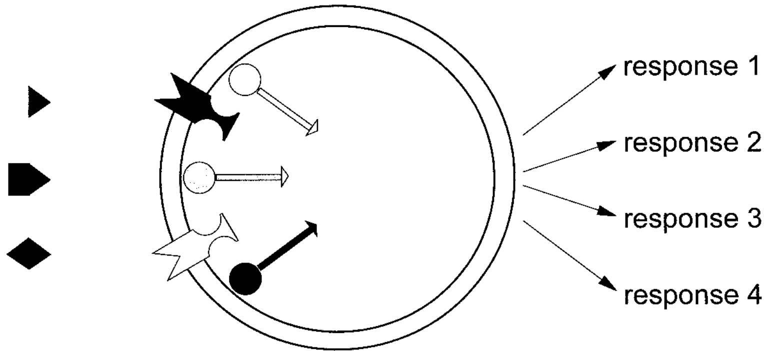


FIG. 6. Model somatostatin signaling system. The system consists of multiple forms of ligands that bind to multiple types of receptors; the receptors are coupled to different effector pathways via multiple types of G-proteins. Structure-function interactions between and among the various components of the signaling system underlie the multiple actions of the somatostatin family of peptides.

processes, from modulation of growth and differentiation to regulation of metabolism, and operate via several modes of action, including autocrine, paracrine, endocrine, and, possibly, semiochemically through distribution in saliva and seminal fluid. The multi-functional nature of this peptide hormone family arises from an elaborate, multi-faceted signaling system consisting of SS signal molecules, G-protein-coupled receptors, and effector pathways (Fig. 6). The most interesting feature of this signaling systems is the substantial diversity at every level. There is structural heterogeneity of the signal itself as well as of the receptor. In addition, the receptors can link to several different effector pathways through numerous different G-proteins. Ultimately, a particular response in a given target cell may be determined by structural interactions between and among elements of the signaling system. For example, by producing and releasing one form of peptide over another, the organism can target specific cells which display receptors that are selective for that peptide form. The response of a target cell also can be modulated by controlling the presentation of receptor subtypes on the plasma membrane as well as by the number and kind of G-proteins and effector systems present. Based upon the distribution of receptor subtypes and the selective binding characteristics of these subtypes, a number of receptor:biological activity correlations are emerging (Table 4).

Our understanding of the SS signaling system is far from complete. Because of the importance of structural interactions between and among diverse elements of the signaling system, future research is needed to understand how the diversity of each level is controlled. For example, what controls the differential processing of PPSSs or the differential expression of SS genes? Similarly, what controls the expression and cellular localization of receptor subtypes, G-proteins, and effector pathway components? Continued research on ligand-receptor and

TABLE 4. *Correlation of somatostatin receptor subtype with physiological action.*

| Receptor | Action | Reference |
|---|---|---|
| 2 | Cognition enhancement | Schettini *et al.,* 1988 |
| 2 | GH inhibition | O'Carrol *et al.,* 1992 |
| 2 | Gastric acid inhibition | Rossowski *et al.,* 1994; Lloyd *et al.,* 1995 |
| 2 | Glucagon inhibition | Rossowski & Coy, 1994 |
| 5 | Insulin inhibition | Rossowski & Coy, 1994 |
| 5 | Amylase inhibition | Rossowski *et al.,* 1994 |
| 1 | Inhibition of gastric smooth muscle contraction | Patel & Srikant, 1994 |
| 5 | Inhibition of cardiac muscle contraction | Feniuk *et al.,* 1993 |

receptor-G-protein interactions also is necessary to explain receptor specificity and effector system linkage. This line of inquiry also may lead to the development of new SS agonists and/or antagonists useful for the enhanced treatment of various human diseases (*e.g.,* tumors and various neurological disorders). Lastly, one of the most compelling questions to be addressed is how did such an elegant and diverse signaling system as the SS signaling system of vertebrates evolve?

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