REVIEW

Novel modalities of somatostatin actions

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Abstract

The first part of this contribution reviews the current knowledge about endocrine and neuromodulatory actions of somatostatin. These biological actions are exerted according to endocrine, paracrine and autocrine modes of action and involve five distinct types of membrane receptors belonging to the 'super-family' of G-protein-coupled receptors.

A new concept concerning a juxtacrine mode of action has recently been introduced to refer to the intervention of cytokines and growth factors in direct, cell-to-cell communication. The evidence in favor of juxtacrine actions of somatostatin will be presented in the second part of this review and illustrated by our own results on macrophage–lymphocyte T interactions in the immune system and spermatogonia–Sertoli cell interactions in mammalian testis. Another phenomenon such as ligand-induced somatostatin receptor homo- and hetero-dimerization resulting in 'poly'-receptors (with characteristics different from those of each of the two receptors forming the complex) is also at the origin of a novel mode of somatostatin action. The latter will be illustrated by the data obtained on human pituitary adenomas with somatostatin analogs specific for either 'poly'-receptor or relevant individual receptors.

The arguments in favor of the analogous mode of actions among different families of chemical messengers such as peptides, cytokines and growth factors are discussed in the concluding section. The emerging unifying concepts on such functional analogies might provide a useful basis for the development of synthetic analogs not only for bioactive peptides but also for other types of chemical messengers.

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Introduction

Bioactive peptides, cytokines and growth factors represent the major types of chemical messengers. Classically, bioactive peptides designate peptidic hormones and neuromediators. Cytokines are mediators of the cell communication in the immune system whereas growth factors represent signals controlling cell division and/or differentiation notably during embryonic induction. Distinction between these three families of polypeptides/glycoproteins is more historical and didactic than functional. Indeed, there is often no clear distinction between the three families and, moreover, the same peptide might functionally belong to all three families.

In addition, natural peptides, cytokines and growth factors have pleiotropic effects and can be released by a multitude of cell types. (For this reason the term 'lymphokines' referring to their lymphocyte origin has been changed to cytokine.) Growth factors and cytokines display 'context'-dependent actions (i.e. their function

depends on the presence of other cytokines and/or their regulators such as binding proteins, soluble receptors or antagonists). They exert specific actions locally in an autocrine, paracrine or juxtacrine manner. In contrast, hormonal polypeptides are secreted into the circulation and transported to the target organs that are located at a distance from their production site. However, it is now well recognized that bioactive peptides also exert autocrine and paracrine actions.

Concerning the relevant receptors, there are slight differences in the way the ligand triggers the initial receptor activation upon binding but the final cellular response is elaborated according to a common, general principle consisting (among other pathways) in activation of different types of protein kinases. For example, cytokine receptors are made by association of a different number of polypeptide chains; most of them have no intrinsic kinase activity and, in this case, signal transduction involves the association of these receptors with cytoplasmic tyrosine kinases. Growth factor receptors are single polypeptide chains with an intrinsic

tyrosine kinase activity. However, the induction of tyrosine kinase activity requires ligand-induced receptor dimerization. In the case of bioactive peptides, receptors are made of a single polypeptide chain spanning the plasma membrane in seven transmembrane domains and are coupled to G-proteins (G-protein-coupled receptors or GPCRs). In resting conditions, G-proteins exist as heterotrimers with GDP bound to their α -subunits. Ligand binding to GPCR triggers a conformational change of GPCR and subsequent activation of a specific G-protein leading to GDP/GTP exchange on G-protein and dissociation of its α -subunit from β/γ -subunits. GTP is hydrolyzed to GDP due to the intrinsic GTPase activity of G-protein thus terminating the cycle of G-protein activation. Dissociated G-protein subunits (i.e. both α and β/γ) activate different transmembrane effectors (e.g. adenylyl cyclases, phospholipases). The effector activation subsequent to ligand–receptor interaction results in a second messenger production and activation of the relevant protein kinases. In all cases, protein kinase-driven phosphorylation results in functional alteration of down-stream effectors (ion channels, enzymes, transcription factors) and an adequate cellular response.

Somatostatin peptide family

The somatostatin (somatotroph release inhibiting factor, SRIF) neuropeptide family comprises few peptides originating from different posttranslational processing of the prepro-SRIF precursor of 116 amino acids. The precursor is encoded by a single gene located in humans on chromosome 3q28. Only two biologically active SRIF isoforms have been identified so far: the tetradecapeptide SRIF-14 and the amino-terminally extended octacosapeptide SRIF-28 (the entire somatostatin-14 sequence is present in the C-terminus of somatostatin-28). Both SRIF-14 and SRIF-28 are found in the periphery and central nervous system (CNS) but the predominant isoform is SRIF-14. The relative proportions of the two isoforms differ among various SRIF-producing tissues. However, SRIF-14 and SRIF-28 display overlapping physiological functions.

The classical physiological functions of SRIF include the inhibition of a wide range of endocrine secretions. Thus, SRIF inhibits the secretions of the pituitary (growth hormone, prolactin and thyrotropin), gastrointestinal tract (cholecystokinin, gastric inhibitory peptide, gastrin, motilin, neurotensin and secretin) and pancreas (glucagon, insulin and pancreatic polypeptide). In the CNS, SRIF acts as a neurotransmitter by itself in distinct, anatomically defined pathways and as a neuromodulator since it has the capacity to modulate the release of other neurotransmitters (among which are serotonin, acetylcholine, glutamate) and neurohormones (e.g. growth hormone-releasing hormone).

Inhibition of various exocrine functions by SRIF is well documented for the secretion of amylase (by salivary glands), hydrochloric acid and pepsinogen (by gastrointestinal mucosa), pancreatic enzymes and bicarbonate (by pancreatic acini) and bile (by liver). SRIF also regulates the intestinal absorption of nutrients and gastro-intestinal motility in a complex manner.

In addition, SRIF has potent immunomodulatory actions on the secretion activity of immune cells such as immunoglobulin (Ig) production by activated B-lymphocytes and cytokine production by activated T-lymphocytes and macrophages. For example, SRIF decreases production of IgA, IgM and IgE by B-lymphocytes and modulates interleukin (IL)-2, IL-4, IL-10 and interferon- γ secretion by T-lymphocytes. Furthermore, somatostatin is apparently involved in the regulation of the Th1/Th2 pattern of cytokine secretion by murine T-cell lines. SRIF also inhibits the secretory responses of some other types of immune cells such as IgE-stimulated histamine and inflammatory cytokines (tumor necrosis factor- α , IL-1 β and IL-6) released from basophils and monocytes respectively. SRIF is also involved in inhibition of neutrophil's chemotaxis, phagocytic activity of monocytes/macrophages and natural killer cell activity.

The proliferative response to antigens/mitogens of different types of immune cells is modulated by SRIF in a complex manner. Indeed, the observed actions on B- and T-lymphocyte proliferation are multimodal: both inhibitory and stimulatory effects have been reported depending on the SRIF concentration, the way in which the immune cell activation was achieved and on the cell phenotype (normal versus malignant) (for review, see 1).

Another peptide, which does not belong *strictu sensu* to the SRIF peptide family but shares 11 amino acids with SRIF, has recently been characterized and named cortistatin (CST). The biologically active form of CST is the tetradecapeptide (CST-14) in rodents and the heptadecapeptide (CST-17) in humans, corresponding to rodent CST-14 amino-terminally extended by 3 amino acids. The CST gene maps to chromosome 4 and 1p36 in rodents and humans respectively. In contrast to SRIF, CST appears predominantly to be confined to the CNS and in particular to the inhibitory inter-neurons of the cerebral cortex and hippocampus. The biological actions of CST involve the regulation of sleep-phase transitions, consolidation of short- and long-term memory and locomotor activity (2). Among human peripheral tissues studied, the highest CST expression has been reported in kidney and testes (3) but CST has also been found in pancreas (4) and in hepatocellular carcinoma cells (5). In the immune system, CST is expressed in human T- and B-lymphocytes (6) as well as in monocytes and monocyte-derived cells such as macrophages and dendritic cells (7). It is currently believed that CST exerts at least some of its biological effects by acting as

an endogenous ligand on SRIF receptors (for review, see 8). This has, in particular, been suggested for the cells of the human immune system (9). However, it has recently been discovered that the orphan MrgX2 receptor binds CST in a highly specific and efficient manner (10), thus suggesting that CST exerts its effects not only by interacting with SRIF receptors but also through its own receptors.

Somatostatin (sst) receptor family

Molecular characterization of rodent and human SRIF receptors indicated that five different receptors underlie the biological actions of the peptide. They are encoded by five different genes and are highly conserved between species (e.g. sst1 of mouse and human share 99% of the amino acid sequence) and within the same species (different SRIF receptors present 45–67% identity between each other). Human genes encoding the sst1, sst2, sst3, sst4 and sst5 receptors have been mapped to 14q13, 17q24, 22q13.1, 20p11.2 and 16p13.3 respectively. Their expression products correspond to monomers composed of 391 (sst1), 369 (sst2), 418 (sst3), 388 (sst4) and 383 (sst5) amino acids. Among these receptors, sst2 appears unique since, in mouse, it can be differentially spliced upon transcription giving rise to two splice variants of 369 (sst2A) and 346 (sst2B) amino acids.

All five somatostatin receptors have been identified throughout the CNS, endocrine and exocrine glands (Table 1). The expression of mRNA for the five cloned receptors is overlapping but the combination (profile) of expression is tissue- and cell type-specific. In more detail, rodent CNS mRNAs for all five types of SRIF receptors have been seen in the cerebral cortex, striatum, hippocampus, amygdala, olfactory bulb and pre-optic area (mouse (11); rat (12)). Among different types of receptors, sst1 and sst2 are the most abundantly expressed whereas the expression of sst3, sst4 and sst5 appears less important. Transcripts for all five receptor types have been visualized in rat but not in human pituitaries where sst4 mRNA is not expressed. Rat and human pancreas, stomach, duodenum, jejunum and ileum contain the transcripts for sst1–sst5 receptors. Other peripheral organs and tissues express SRIF receptor transcripts more selectively. For example, rat adrenals and testes contain sst1–sst3 transcripts. Elevated expressions of sst3 in liver and spleen and of sst4 in lung, heart and placenta have been seen. The expression of sst2A and sst3 receptor mRNAs has been reported in cells of the immune system such as activated macrophages, and T- and B-lymphocytes (for reviews see 1, 13, 14).

Both biologically active forms of SRIF (SRIF-14 and SRIF-28) recognize the five cloned receptors with similar affinities, but somatostatin-28 binds to sst5 receptors with slightly (tenfold) better affinity. Two subfamilies of somatostatin receptors are distinguished

based on their affinity for routinely used analogs. Thus, octreotide (SMS 201-995), vapreotide (RC 160) and lanreotide (BIM 23014) distinguish sst2 > sst3/sst5 from sst1/sst4 since they bind to the sst2/sst3/sst5 subfamily with high-to-moderate affinity and binding is 1000-fold less efficient to the sst1/sst4 subfamily of receptors. However, more recently a series of receptor subtype-selective analogs has become available (Table 1). In addition, a new agonist (SOM230) with high affinity for sst1, sst2, sst3 and sst5 and with a lower affinity for sst4 has also been synthesized. SOM230 thus appears as a 'universal' ligand for all types of SRIF receptors (15) which, in this context, is similar to the natural peptide.

All five cloned somatostatin receptors (sst1–sst5) belong to the superfamily of G-protein-coupled receptors with seven transmembrane domains. The activation of G-proteins upon somatostatin binding is, however, coupled to multiple signaling pathways (Table 1). After expression of the cloned receptors in heterologous cell lines, all of them (sst1–sst5) inhibit the adenylyl cyclase activity (through $G\alpha_{1-3}$) but, in parallel, also selectively activate other transduction effectors such as phospholipase-C β (through both pertussis toxin-dependent and -independent G-proteins). In addition, SRIF receptors activate several types of ionic channels (inward rectifying K⁺-channels and high voltage dependent Ca²⁺-channels). A number of phosphatases such as serine/threonine phosphatases, tyrosine phosphatases and Ca²⁺-dependent phosphatases are also involved in transduction of SRIF actions. Effectors such as extracellular regulated kinase (ERK) 1/2 and Na⁺/H⁺-exchanger also mediate SRIF-induced cellular responses (13, 16, 17).

The question concerning the selective involvement of different somatostatin receptors in specific physiological responses to SRIF is still a matter of debate. However, some precise physiological functions could be attributed to each of the five known receptors. The majority of the relevant data came from the studies on knock-out (KO) mice in which gene encoding for a given somatostatin receptor has been invalidated. These animals are now available for each of the five receptors (e.g. for sst1–sst4 (18) and for sst5 (19)) but the most studied remain sst2 and sst5 KO mice. The studies of sst2 gene KO mice indicated that this receptor displays specific central actions such as fine motor control (20), modulation of spatial learning (21), exploratory activity and emotional reactivity (22). Among the peripheral actions of SRIF, sst2 receptor appears specifically involved in the inhibition of gastric acid secretion (23, 24). Analysis of the sst5 KO phenotype showed that this receptor mediates SRIF-induced inhibition of insulin secretion and glucose homeostasis (19) in an age-dependent manner (25).

Certain physiological roles of somatostatin receptors that had initially been suggested based on their colocalization with hormones have therefore been

Table 1 Characteristics of somatostatin receptors.

	sst1	Reference	sst2	Reference	sst3	Reference	sst4	Reference	sst5	Reference
Ligand selectivity	L-797591	76	L-054522	69, 76	L-796778 BN-81644 BN-81674	76, 79	L-803087 NNC-269100	76, 80	BIM-23268	74
			L-779976	77, 78						
Signal transduction										
Adenylyl cyclase	–	81, 82	–	83, 84, 78	–	85, 86, 87	–	88, 89	–	90, 91
Ca-channels	–	92	±	93, 94, 95, 96					–	95
K-channels	–	97	+	98	+	98	+	98	+	98
Phospholipase C	+	82, 99	+	99, 100, 101	+	99, 102	+	99, 103	±	99, 103
MAP kinase	+	104	–	77, 105	–	106	+	88, 107	–	38
Tyrosine phosphatase	+	35, 108	+	35, 36, 77, 109	+	110	+	110	+	111
Na-proton antiporter	–	112, 113	±	113			+	114		
Expression – central										
Forebrain	+	115	+	115	+	85, 115	+	115, 116	+	117
Hypothalamus	+	8, 115	+	8, 115	+	8, 115	+	8, 115	+	8, 90
Cerebellum			+	115	+	115	+	115	+	115, 118
Expression – peripheral										
Lung	+	116					+	116		
Heart									+	118
Skeletal muscle									+	118
Stomach	+	119, 120	+	119, 121	+	119	+	119	+	119
Kidney	+	116	+	120, 122						
Liver	+	116								
Pancreas	+	63, 116	+	63	+	63, 85, 116	+	63	+	63
Adrenal									+	118

confirmed by analysis of the relevant receptor KO phenotype. This is the case for the explicit demonstration coming from KO mice concerning sst5 receptor involvement in the regulation of insulin secretion seen also in the histochemical studies by Mitra *et al.* (26) and Ludvigsen *et al.* (27). In other cases, as for example concerning the specific roles of the sst1 receptor in the retina, the KO model was less informative. Indeed, it has been reported that the genetic deletion of sst1 receptor triggers an over-expression of sst2 receptors and vice-versa (28). This phenomenon of the co-regulation of sst receptor expression particularly hampered the analysis of data obtained in KO models in relation to the involvement of a given receptor type in the physiological effect of SRIF known to implicate multiple receptors. For example, the involvement of sst1 receptor in the regulation of growth hormone (GH) secretion that has been documented in sst1-null mice (29) should now be reconsidered in the light of the co-regulation of sst1 and sst2 receptor expression (28), especially because sst2 receptor has also been involved in the regulation of GH secretion in both rodent (30) and human (31) pituitaries.

Somatostatin and cancer

The capacity of SRIF to negatively regulate cell proliferation through both indirect and direct mechanisms has repeatedly been reported. Indirect actions include the inhibition of secretion of growth-promoting hormones and growth factors stimulating the growth of various cell types. For example, the ability of SRIF to inhibit insulin-like growth factor-I expression and serum levels represents the rationale for its use in an adjuvant therapy for tamoxifen in the treatment of breast cancer (32). However, it should be stressed that the recent clinical trials (phase III) indicated that adding somatostatin analogs to tamoxifen do not increase the therapeutic benefit of tamoxifen in the treatment of advanced breast carcinoma (33). Besides, the capacity of SRIF to inhibit angiogenesis is of potential clinical relevance for the indirect control of tumor cell growth (16, 34).

The ability of SRIF to control cell number by both inhibiting cell division and triggering programmed cell death by apoptosis has been demonstrated *in vitro*. Thus, it has been shown that SRIF inhibits the proliferation of cell lines transfected to express sst1, sst2 or sst5 receptors (35, 36). The underlying mechanisms involve the activation of SHP tyrosine phosphatases and ERK pathways (35, 37, 38). The induction of apoptosis by SRIF in the cell lines studied is mediated by sst2 (39) and sst3 (40). SRIF-mediated inhibition of cell division and the triggering of cell death are the features that are currently used therapeutically in order to achieve a direct control of tumor growth by this peptide. Obviously such an approach is only possible in those cancers expressing SRIF receptors. This is the case for

neuroendocrine, gastro-entero-pancreatic, brain, breast, prostate and lung cancers. In addition to the relevant therapeutic applications, a SRIF analog such as indium¹¹¹-labeled octreotide is routinely used for diagnostic purposes in cancers displaying SRIF receptors. SRIF receptor scintigraphy has indeed proven valuable in the detection of a range of human cancers and their metastases (41).

It should be stressed that more than 80% of the carcinoid tumors studied display sst2 and sst5 receptors (sst5 being less abundant than sst2) (for review, see 16). Besides, such a pattern of receptor expression by tumors brings, *a posteriori*, the rationale for the efficiency of anti-cancerous SRIF analogs (octreotide, lanreotide, vapreotide). The latter analogs had been developed before the molecular cloning of SRIF receptors, based on their high efficiency to inhibit SRIF-relevant hormone secretions and their resistance to enzymatic degradation after subcutaneous administration (for historical overview, see 42). However, it should be kept in mind that the level and pattern of expression of SRIF receptors vary greatly among different carcinomas. Indeed, the efficacy of clinically used SRIF analogs has been explicitly proven only for the treatment of neuroendocrine tumors. In addition, the sst2-preferential radiolabeled SRIF analogs, such as ¹⁷⁷Lu-octreotate, appear very promising for the treatment of neuroendocrine gastro-entero-pancreatic tumors (43). Importantly, the loss of a specific type of SRIF receptors (sst2 in particular) has been observed in tumors other than those of neuroendocrine origin (e.g. pancreatic and prostatic adenocarcinomas). It might explain the lack of therapeutic benefit of clinically used SRIF analogs in these pathologies (44). Altogether, these data emphasize the need for further development of new SRIF analogs (additional to sst2/sst5 preferential analogs) for the purpose of anti-cancerous therapies. More generally and considering the new modalities of somatostatin actions in terms of receptor dimerization discussed below, development of hybrid somatostatinergic molecules appears particularly promising in the perspective of clinical treatment of pituitary tumors (adenomas). Indeed, almost all GH-secreting adenomas express sst2 and sst5 receptors whereas prolactinomas (i.e. prolactin (PRL)-secreting adenomas) express predominantly sst5 receptor. Although octreotide and lanreotide have been shown to be able to suppress GH secretion successfully in GH-secreting adenomas some of these tumors are refractory to this treatment as are the majority of prolactinomas. The development of sst2/sst5 bi-specific analogs on the one hand, and of somatostatin/dopamine hybrid molecules on the other hand, pointed to their great efficacy to suppress *in vitro* GH secretion from refractory GH-secreting adenomas and PRL secretion from prolactinomas respectively (reviewed in 45, 46). The example of adenomas, therefore, nicely illustrates how the integration of the new concepts on

modalities of somatostatin actions (c.f. below) in strategies used for the development of new SRIF analogs might be a powerful tool for the achievement of the better control of the size and hypersecretion from sst receptor-expressing neuroendocrine tumors.

New modalities of somatostatin actions: juxtacrine effects

Such a plethora of physiological and pathological effects of SRIF are mediated through classical (neuro)-hormonal, paracrine and autocrine pathways. A growing body of evidence now suggests that SRIF may also act in a juxtacrine manner, through a variant of cell-to-cell interaction. For example, during antigen presentation, an antigen presenting cell (APC) such as a macrophage is very close to the CD4⁺ T-lymphocyte. This spatial proximity is a consequence of T-cell receptor (TCR) interaction with antigenic peptide presented in the context of major histocompatibility complex (MHC) class II molecule occurring simultaneously to CD4⁺ interaction with MHC class II molecule. In mice, activated macrophages are producing SRIF (47) whereas T-lymphocytes express SRIF receptors (48). Thus, intimate contact between the two cells during the process of antigen presentation may allow SRIF to act on T-lymphocytes in a juxtacrine manner. Interaction of SRIF with its receptors is sufficient to trigger

the cytokine production by T-cells (49). SRIF might therefore directly induce the secretory response of activated T-cells. In addition, SRIF might modulate the antigen-induced response (proliferation and cytokine secretion) by either potentiating or attenuating it (for review see 1). In consequence, SRIF secreted by APC should be considered as a costimulatory signal acting through a juxtacrine mode in a fashion similar to the B7 ligand expressed on APC plasma membrane. Consistently, SRIF receptors (expressed on the plasma membranes of T-lymphocytes) could be seen as functionally analogous to the costimulatory receptor CD28 (Fig. 1). It should, however, be noted that in humans, although the principle of the costimulatory role exerted through SRIF receptors appears applicable, the ligand responsible for activation of these receptors is CST rather than SRIF itself. Indeed, we (50, 51, 52) and others (53, 54) were able to identify different types of sst receptors (with consensus being reached for sst3) on human normal and leukemia T-lymphocytes. But, in contrast to murine macrophages, human macrophages and dendritic cells express CST (6, 7).

Another example of juxtacrine actions of SRIF is seen in mammalian testes. Given the intimate contact between Sertoli cells and male germ cells, it has been hypothesized that regulatory factors produced by Sertoli cells might act on spermatogonia through a juxtacrine mode of action and vice-versa. The recently

Figure 1 A model according to which somatostatin (SRIF) receptors (sst2) might be considered as T-lymphocyte receptor for costimulatory signal SRIF through a juxtacrine communication between murine T-lymphocyte and macrophage. Interaction between SRIF produced by antigen presenting cell (APC, here activated macrophage) and its receptor sst2 inhibits (⊥) adenylyl cyclase (AC). This inhibition might be additive in terms of a decrease in intracellular cAMP concentration to that subsequent to the activation of phosphodiesterase (PDE) after CD28 receptor interaction with B7 ligand expressed on APC plasma membrane. Altogether, these interactions lead to protein kinase A (PKA) inhibition. Given that elevated PKA activity exerts a tonic restraint on lymphocyte proliferation, PKA inhibition results in the release of the blockage. The subsequent increase of proliferation (↑) might synergize with phospho-tyrosine-pathway-dependent induction of proliferation initiated through T-cell receptor (TCR)/antigenic (Ag) peptide interaction on the one hand and CD4 receptor/MHC II (major histocompatibility complex, class II) molecule interaction on the other hand.

demonstrated interaction of Sertoli cells and spermatids (post-meiotic, relatively differentiated male germ cells) through Sertoli cell-derived androgen-binding protein (ABP) which, in turn, triggers the expression of spermatid-specific transcription factors, well illustrates testicular juxtacrine interactions (55). In this context, we have recently reported that in immature porcine testis, spermatogonia (male germ-line stem cells) produce SRIF whereas both spermatogonia and Sertoli cells express sst2 receptors (56, 57). Spermatogonia and Sertoli cells are in intimate contact by which the Sertoli cell provides physical, regulatory and nutritive support to spermatogonia. We were able to demonstrate that such an intimate contact has direct functional consequences on both cell types. Thus, SRIF inhibits stem cell factor (SCF) expression by Sertoli cells concurrently with the inhibition of SCF-induced spermatogonia proliferation (56). In this example, juxtacrine and autocrine actions of SRIF are combined to allow orchestrated inhibition of spermatogonia proliferation through both direct and indirect effects. SRIF-mediated inhibition of spermatogonia proliferation via sst2 receptor underlies the peptide's direct effects in an autocrine manner whereas the inhibition of SCF expression through the same receptor type allows an indirect control of spermatogonia proliferation in a juxtacrine manner. Indeed, SCF is the major mitogenic and survival factor for spermatogonia (58, 59).

The superposition of different modes of action of a single peptide (SRIF) through a single type of receptor (sst2) provides, therefore, a fine-tuning of a given physiological response such as spermatogonia proliferation (Fig. 2). It remains to be determined, however, if the paradigm of juxtacrine actions of SRIF as proposed here for porcine testes might be applicable to sst3, sst4 and/or sst5 receptor interaction with intra-testicular SRIF in human testes. Indeed, we previously reported the expression of these three receptor types in normal human testes (60). In addition, the presence of SRIF in human testicular homogenates has been demonstrated (61) but the precise cell types expressing SRIF are, as yet, unknown.

New modalities of somatostatin actions: receptor dimerization

The concomitant expression of multiple SRIF receptors by a single cell has been well established (e.g. in pituitary (62) and pancreatic (63) cells). By analogy to other types of G-protein-coupled receptors, such co-expression of different types of SRIF receptors raised the possibility for homo- and heterodimerization between them. During the last few years such interactions have indeed been demonstrated, at least in heterologous cell-expression systems such as epithelial and fibroblast cells. Thus, the homodimerization of sst2, sst3 (64) and sst5 (65, 66, 67) has been reported and, at least in the case of sst5, it has been explicitly

Figure 2 A model for juxtacrine interactions between spermatogonia and Sertoli cell via somatostatin (SRIF) in immature porcine testis. Such interaction is plausible because spermatogonia produce SRIF and Sertoli cells express sst2 receptors. Activation of sst2 receptor by SRIF binding leads to a diminished expression of stem cell factor (SCF) expression by Sertoli cells. Because SCF is the main mitogenic factor for these cells, such inhibition of SCF expression is associated with a decrease in spermatogonia proliferation. This juxtacrine regulation of spermatogonia proliferation might be superposed to a putative autocrine control via sst2 receptors expressed by spermatogonia themselves. Indeed, given the anti-proliferative actions of SRIF, the autocrine interaction SRIF/sst2 receptor might directly inhibit spermatogonia proliferation.

demonstrated that it is SRIF induced. However, the homodimerization of either sst1 (68) or sst4 has not been revealed so far. The heterodimerization of sst2 and sst3 (64) as well as of sst1 and sst5 (65, 67) has also been reported. The heterodimerization with other types of G-protein-coupled receptors such as mu-opioid and dopaminergic D2 has been reported for sst2 (69) and sst5 (66) respectively.

These different types of receptor oligomerization lead to the formation of 'poly'-receptors with qualitatively new characteristics in terms of receptor affinity for ligand binding, efficiency of the relevant signal transduction and receptor trafficking. For example, the affinity for the sst3-selective ligand is greatly diminished in the case of the sst2/sst3 complex, it is not altered for the sst2-selective ligand in the case of the sst2/sst3 complex and is greatly enhanced for the sst5-selective ligand in the case of the sst5/D2 receptor complex. Similarly, the effect of receptor oligomerization on adenylyl cyclase and ERK pathways varies greatly depending on the receptor partners. Thus the transduction via sst3 is silenced by its heterodimerization with sst2 (64) whereas the sst2-selective agonists activate the ERK pathway much more significantly through the sst2/mu-opioid receptor dimer than through the monomeric sst2. The latter is evident even if the 'poly'-receptor has a lower affinity for sst2 agonists than the sst2 monomer (69). Finally, at least in some circumstances, the receptor oligomerization alters the dynamics of receptor internalization. For example, sst5-selective ligand-induced internalization of sst5 receptor is decreased when it is complexed with sst1 (67). Similarly,

sst3 is not internalized by sst3-selective ligands when it is dimerized with sst2 (64).

The new characteristics conferred to SRIF 'poly'-receptors by oligomerization of the individual sst receptors might become valuable pharmacological tools. Indeed, a synthetic bi-specific ligand BIM-23244 (i.e. sst2 and sst5 preferential) is able to inhibit GH secretion by somatotrope adenomas with an affinity better than that of octreotide. Moreover, this agonist is efficient even in octreotide-resistant adenomas probably because sst5 'prolongs' the action of sst2-selective component on the sst2/sst5 heterodimer due to the slower internalization and the faster re-sensibilization than the relevant sst2 kinetics (70). Similarly, chimeric SRIF/dopaminergic molecules (BIM-23A387) acting apparently through sst2/D2 receptor dimers are more potent in suppressing GH and PRL secretions from human pituitary somatotrope adenoma cells (71).

The combinatorial engagement of different receptor subtypes by SRIF itself or by chimeric ligands contributes to the increase in diversity of the cell receptor equipment and increases the molecular basis for the pleiotropy of SRIF biological actions (Fig. 3). However, it should be kept in mind that homo- and heterodimerization of SRIF receptors has so far been documented only in cell lines and that both remain to be established in non-transformed cells. In this context, the data of Baragli and co-workers (72) pointing to the heterodimerization of dopamine D2 receptor and somatostatin

sst2 receptors in primary human astrocytes appear very relevant.

New avenues of specificity

The specificity of SRIF actions on a particular cell is achieved through a combination of receptor isoform expression by the cell in question. An increasing body of evidence now suggests that this specificity can be modulated by different factors since the pattern of receptor isoform expression displays some degree of plasticity. Schematically, the factors impinging on the specificity of SRIF actions may be considered as temporal, environmental and functional.

The recently documented shift from sst2A to sst3 receptor expression in maturing thymocytes represents an example of the putative temporal correlates of SRIF specificity (73). Indeed, immature CD2 + CD3- thymocytes are actively proliferating cells in the process of differentiation via TCR rearrangement, whereas mature, differentiated CD3+ thymocytes undergo apoptosis during positive and negative selection occurring in this primary lymphoid organ. The observed developmentally regulated shift in sst-receptor expression therefore suggests that SRIF might be involved in CD2 + CD3- cell differentiation through the sst2A receptor while it might regulate CD3 + cell selection by apoptosis via sst3 (73).

Modulation of SRIF specificity by environmental factors has not been investigated yet either in non-transformed cells (*in vitro*) or *in vivo*. However, based on the data on SRIF receptor oligomerization obtained in cell expression systems, it is reasonable to assume that the simultaneous presence of other ligands (e.g. dopamine, opioids) and activation of other GPCR receptors by these ligands (e.g. D2-dopaminergic, μ - and δ -opioid receptors) might be the origin of subsequent receptor oligomerization, thus conferring a different pattern of receptor engagement and different functional read-out of SRIF actions depending on the presence or absence of other ligands (Fig. 4). These considerations, if confirmed by experimental evidence *in vivo*, would have important conceptual implications. In this case, the concept of specificity of a given ligand depending on the pattern of the relevant receptor expression should then be enlarged on the pattern of differential receptor engagement in the presence of different combination of other ligands.

Finally, the functional state of the cell is also responsible for the alteration of SRIF specificity. For instance, both normal and tumoral pituitary lactotropes express sst2 and sst5 receptors. However, prolactin secretion is predominantly inhibited by, respectively, sst2 and sst5 receptor in normal embryonic and tumoral lactotropes (74, 75). Therefore, in the course of pathogenesis (i.e. transition from physiological versus pathological states in a particular cell) there might also be a shift in the type of receptor involvement in a

Figure 3 Receptor oligomerization as a source of diversity. Receptor dimerization may be a consequence of SRIF binding to either different SRIF receptor isoforms (sstx and ssty) or can result from dimerization of sstx receptor and another GPCR (Dx) activated by another ligand (DA). In both cases receptor interaction leads to the alteration in receptor affinity, trafficking and signal transduction. The so formed 'poly'-receptor has the qualitatively novel characteristics that might explain the fact that a small number of SRIF receptor isoforms mediates a panoply of SRIF actions.

Figure 4 New avenues of SRIF specificity. The specificity of SRIF actions might be affected by environmental, temporal and functional parameters. Environmental factors that might alter the specificity involve, for example, the presence of another ligand (X) capable of activating receptors that can dimerize (i.e. dopaminergic, opioid) with sst receptors. In this case, the specificity of SRIF actions mediated by receptor heterodimer would be different from that resulting from exclusive activation of sst receptor by SRIF. Temporal parameters involve, for example, a developmentally regulated shift from one (sst2A) to another (sst3) receptor isoform expression during thymocyte maturation from CD2+CD3- to CD3+ cells. Finally, in some pathological states, the same SRIF action (e.g. inhibition of prolactin secretion) might be mediated by a receptor (sst5) which is irrelevant in the physiological state.

given cellular response (i.e. prolactin secretion in this example). Such a shift might occur without obvious alteration in the pattern of receptor expression by the cell (Fig. 4).

Overall, the modulation of the effects of SRIF by temporal, environmental and cell functional state factors leads to the additional diversification of its specificity.

Conclusions

Some novel physiological actions have been discovered for the natural peptides such as SRIF during the last two decades. Indeed, it is now well accepted that they are produced by pathogen-activated immune cells (e.g. macrophages) and subsequently activate other types of immune cells (e.g. T-lymphocytes) in an orchestrated succession of cell-to-cell interactions inherent to the immune system and indispensable for mounting an efficient immune response. Natural peptides are therefore the functional equivalent of cytokines, the latter being, until recently, considered as unique immunopeptides. Indeed, these two classes of chemical messengers share different modes of action (autocrine, paracrine and, more recently demonstrated, juxtacrine). More importantly, these modalities of action appear general (i.e. not limited only to the immune system) considering, for example, SRIF actions in spermatogonia–Sertoli cell communication in testes.

In addition, the ligand-induced receptor dimerization, until recently considered as exclusive for, and inherent to, the activation of cytokine and growth factor receptors, has now been demonstrated for SRIF-induced receptor oligomerization. Thus oligomerization also appears as a general principle of receptor activation, irrespective of the class of receptor and the nature of the ligand. In the particular case of SRIF, the ligand-induced formation

of receptors of higher order should now be assessed systematically in all of its physiological targets. Indeed, virtually all of them co-express more than one sst receptor and obviously express other types of G-protein-coupled receptors.

In conclusion, these 'novel' modalities of SRIF action in terms of juxtacrine actions and/or receptor oligomerization contribute to increase the functional diversity of its physiological effects and might further help to explain how a limited number of receptors might underlie a plethora of its biological roles. We are convinced that the better understanding of such 'novel' modalities of SRIF actions would soon become a paradigm for the discovery of similar modalities of action for other natural peptides that, in time, might open new therapeutic avenues.

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