Novel chimeric somatostatin analogs: facts and perspectives

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Abstract

Dopamine and somatostatin receptor agonists inhibit hormone secretion by normal pituitary cells and pituitary adenomas. Indeed, initially several dopaminergic drugs, and lately somatostatin analogs, have been developed for the treatment of pituitary adenomas. Recently, it has been demonstrated that subtypes of somatostatin and dopamine receptors may form homo- and hetero-dimers at the membrane level, as part of their normal trafficking and function. Interestingly, a specific ligand for a given receptor may influence the activity of an apparently unrelated receptor, and the association between the two different receptors could be induced by addition of either dopamine or somatostatin. The new properties of these families of G-protein coupled receptors (GPCRs) offer a potential explanation for the apparent conflicting results observed both in vivo and in vitro in human cell systems treated with the presently available analogs. Moreover, this observation not only increases the possibilities of modulating the activities of these receptors, but also raises new questions on the role of associations of specific receptors in the control of cell functions. In fact, results from preclinical studies have shown that receptor activation may not only trigger different intracellular signaling pathways, but also induce a distinct response depending upon the specific cell type. Recently, a number of new interesting compounds (subtype selective analogs and antagonists, as well as bi-specific and hybrid somatostatin/dopamine compounds) have been developed. The effects of these new molecules have been explored in few animal and human cell lines and primary cultures from human tumors, revealing a heterogeneous, but broader, profile of activities. Further studies are certainly needed to fully elucidate the complex interplay between the GPCRs and consequent biological effects, to identify suitable therapies for controlling hormonal secretion of pituitary tumors. However, these recent observations form the basis for the application of new interesting strategies for the treatment of not only pituitary tumors but also other human malignancies.

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Dimerization of G-protein-coupled receptors (GPCRs)

GPCRs are among the most common gene products in mammals and regulate several cell functions. Until a few years ago, each receptor was believed to interact separately with G proteins, but formation of dimers, or even oligomers, as may happen with tyrosine kinase growth factor receptors, was not supposed to occur for GPCRs. A series of observations have challenged this conclusion, beginning with bivalent antibodies directed against a peptide antagonist of the gonadotropin-releasing hormone receptor and with the finding of a functional reconstitution of receptor activities occurring when two mutated and inactive angiotensin receptors were co-expressed (reviewed in Refs (1–4)). Recently, biophysical methods based on luminescence and fluorescence energy transfer have confirmed the existence of such oligomeric complexes in living cells (1–3). Although their existence is now largely accepted, their functional importance remains more enigmatic and in some cases even controversial. Heterodimerization between distinct GPCRs could be responsible for the generation of pharmacologically defined receptors for which no gene has been identified so far. Although the majority of GPCRs were demonstrated to form constitutive dimers, perhaps during biosynthesis, and the formation of a constitutive dimer is dependent on receptor density, several reports in the literature suggest that GPCR dimers are not only constitutively present, but also ligand promoted (2). However, it should be noted that in some studies where ligands were apparently found to promote dimer
Biological and clinical significance of somatostatin and dopamine receptor (DR) dimerization

Although GPCR dimerization is not common, in contrast with dimerization of tyrosine kinase receptors and cytokine receptors, several GPCRs, including specific subtypes of somatostatin and DRs (Table 1), are now known to form homo- and hetero-dimers (5). The somatostatin receptor (SSR) family contains five subtypes (somatostatin receptor subtypes 1–5, sst1–5) that are activated by the natural ligands somatostatin-14 and somatostatin-28 (Fig. 1) as well as cortistatin (6). These receptors have been found in a number of different tissues and play key roles in regulating hormone release, endocrine and exocrine function, gastrointestinal motility and secretion, sleep, cognition, and, to a certain extent, cell proliferation. SSRs regulate diverse signal transduction pathways, such as adenylyl cyclase and guanylyl cyclase inhibition, phospholipase C and phospholipase A2 stimulation, tyrosine phosphatase activation, or ionic conductance channels modulation (6). Recently, particular attention has been focused on the receptor-mediated mechanisms involved in the control of cell differentiation or growth inhibition and specific intracellular pathways have been elegantly characterized (7–10). Moreover, novel findings regarding receptor expression regulation and trafficking have opened new opportunities to unravel unsolved questions on SSR pathophysiology and expand the applications of new somatostatin analogs in the management of human tumors. For example, the recent knowledge on receptor internalization and recycling may solve a number of problems occurring with tumor imaging as well as SSR-targeted radiotherapy, while the latest data on the differential intracellular sorting of SSRs may provide important clues about the regulation of receptor responsiveness during long-term administration of somatostatin analogs (11, 12).

The five SSR subtypes are known to form homo- and hetero-dimers in specific combinations (13–15). For example, sst5 associates robustly with sst1, but not with sst4 (13, 16, 17). The sst1/sst5 interaction seems to affect the ligand-binding and internalization properties of the receptors (13, 17). Indeed, sst1 does not undergo efficient agonist-promoted internalization when expressed alone, but does undergo significant endocytosis when co-expressed with sst5 (13). These new findings are crucial for better understanding the agonist-dependent desensitization and internalization of those SSRs mainly involved in regulation of important cell functions. For example, receptor dimerization may influence the receptor coupling to adenylyl cyclase, which is dependent on the length of the C-tail, whereas desensitization and internalization require specific structural domains. The C-tail of sst5 serves a multifunctional purpose in mediating effector coupling, agonist-dependent desensitization, and internalization (18). How this component of the receptor is involved in the dimerization is still unknown; however, it may be critical for the intracellular fate of the protein. Since sst5 is one of the principal SSR subtypes in endocrine tissues, elucidation of the molecular signals underlying these processes will provide a better understanding of the function of this receptor during prolonged agonist treatment normally and in disease, such as pituitary and gastroenteropancreatic (GEP) neuroendocrine tumors (NET). The performance of sst2 and sst1 that

![Table 1 Dimerization of somatostatin and dopamine receptors.](www.eje-online.org)
specifically heterodimerize with each other but have not been shown to interact with other SSR subtypes is also of interest (14). Indeed, an important functional consequence of the sst2/sst3 interaction is a marked alteration in the desensitization profile of the heterodimer relative to that of the individually expressed receptors (14).

Importantly, SSR subtypes interact not only with each other (Fig. 1), but also with other GPCRs. Indeed, sst2 has been found to interact with the μ-opioid receptor (19). This association has no evident effect on the ligand-binding properties of the receptors, but does allow for significant co-internalization and cross-desensitization (19). Conversely, sst3 exhibits heterodimerization with the D2 dopamine receptor (D2R), and the sst3/D2R heterodimer displays enhanced signaling and altered pharmacological properties relative to the individually expressed receptors (20). Indeed, when sst3 and D2R form functional heterodimers, they reciprocally influence their function and binding of either receptor increases the ability to inhibit cAMP production by the other receptor in response to its own ligand (19). Moreover, the dynamic association between the two different receptors could be induced by addition of either dopamine or somatostatin. The discovery of this phenomenon may help to explain the well-known examples of synergistic interactions between somatostatin and dopamine in the CN and the pituitary (21–23).

Physical interaction has been described for different SSR subtypes, and although the specific site of interaction has not yet been discovered, this phenomenon opens a new scenario to interpret results obtained in vivo or in isolated cells that are in contrast with the classical binding and activation pathways described for individual receptors. This observation raises the possibility of a new level of complex interactions between receptors that could multiply the range of activities of the already large family of GPCRs. Recent results from preclinical studies have shown that receptor activation may not only trigger different intracellular signaling pathways, but also induce a distinct response depending upon the specific cell type. In view of these novel findings on GPCRs, a number of new interesting compounds, subtype selective analogs and antagonists, as well as bi-specific and hybrid somatostatin/dopamine compounds, have been developed. The effects of these new molecules have been explored in animal and human cell lines and primary cultures from human tumors, thereby revealing a broader profile of activities and forming the basis for the application of new strategies for the treatment of human malignancies.

**Figure 1** Somatostatin and dopamine receptor subtypes membrane expression and their endogenous ligands, somatostatin-14, somatostatin-28, and dopamine (top). Example of formation of heterodimers between somatostatin and dopamine receptor subtypes co-expressed on the same cell and change in intracellular signaling. Chimeric compounds are molecules that combine structural elements of both somatostatin and dopamine and may retain affinity for specific somatostatin and dopamine receptor subtypes (bottom).
abundantly express D2R and to a lesser extent sst5, there is an established dominant role of sst5 in controlling PRL release by selective somatostatin analogs (24). Although the significance of the association with sst1 and D2R expression is still unknown, the recent availability of new subtype-selective, bi-specific, universal, and hybrid compounds is progressively offering the possibility to characterize receptor activities in specific cells, as well as receptor interaction in cells constitutively expressing SSRs and DRs. Indeed, the bi-specific somatostatin analog, BIM-23 244, targeting sst2 and sst5, has been demonstrated to induce a greater in vitro growth hormone (GH)- and PRL-suppressive effect on GH/PRL-secreting pituitary tumors partially responding to classical somatostatin analogs (25). However, whether this type of strategy, using sst2 and sst5 agonists, might also be applied to other target cells remains to be established because, as previously mentioned, different cells with a similar receptor profile may differentially respond to the same ligand-induced receptor activation.

These new compounds also seem helpful to establish precisely the synergistic or additive effects, which can be observed in vivo during therapy with somatostatin analogs and dopaminergic drugs in patients with pituitary adenomas. Indeed, the lack of additive effect of an sst2-preferring agonist (BIM-23 023) and a D2R-preferring agonist (BIM-53 097) in suppressing GH and PRL release has been observed in cultured human pituitary adenoma cells from patients who were both fully and partially responsive to somatostatin analogs (26). Conversely, an enhanced potency in suppressing hormone secretion, as compared with either single or combined administration of somatostatin analog and dopamine agonist, has been noticed in the same cases when a new chimeric molecule (BIM-23A387) was used (26). Most of the tumors that are partially responsive to octreotide may better respond in vitro not only to the bi-specific sst2/sst5 or the chimeric sst2/D2R ligands, but also to the sst2/sst5/D2R hybrid ligands (Fig. 1), such as BIM-23A760 (27). When compared with universal SSR ligands, this latter compound seems more efficacious in the inhibition of GH secretion from cell cultures of pituitary adenoma (28). However, the variable patterns of response to sst2, sst5, and D2R analogs may explain the greater efficacy of BIM-23A760, which bind to the three receptors, in suppressing GH secretion. This evidence suggests that the use of targeted drugs, according to specific receptor expression and functionality, which may vary among the various classes of tumors, represents perhaps the correct future strategy for the clinical applications.

These findings again strongly support the hypothesis that these new compounds may induce GPCRs dimerization, or interact with pre-existent receptor dimers, or even modulate alternatively receptor trafficking and desensitization. Interestingly, it has also been shown that in adenomas displaying different expression profiles of sst2, sst5 and D2R, treatment with the sst2-selective agonist or the sst2/sst5 dual agonist significantly inhibited GH secretion, whereas treatment with the sst5-selective agonist did not modify the secretion activity of the adenoma cells when D2R was co-expressed (29). Conversely, the expression of sst5 seems to confer a higher sensitivity of the somatotrope adenoma cells to the antitumor activity of somatostatin analogs dissociated by the antisecretive one (30, 31). Indeed, the role of sst2 and sst5 in modulating cell proliferation and hormone secretion in pituitary adenomas is not completely understood, and once again it is not only the specific receptor profile, but also the targeted cell that seems to determine the final outcome of the treatment. In fact, in vitro cultures of human clinically non-functioning pituitary adenomas (NFA), but secreting α-subunit, sst2-selective agonists inhibited hormone secretion without affecting cell viability, whereas sst5-selective agonists did not influence secretion but unexpectedly promoted cell viability (32). Similar to NFA, sst3-selective agonists also stimulated cell proliferation in a medullary thyroid carcinoma cell line, where sst2-selective agonists exerted an inhibitory effect (33). Other authors have evaluated the effect of several SSR-selective agonists, BIM-23A387, and bromocriptine on a very heterogeneous series of NFA in vitro and found that all these compounds may affect the viability of some, but not all, tumor cells, exerting roughly a similar potency (34).

That the chimeric compounds could produce a greater PRL suppression in prolactinomas resistant to dopaminergic drugs and expressing high levels of SSRs remains to be established. Conversely, a predominant expression of sst5 has been described in corticotrope adenomas, as well as the corticotrope cell line AtT-20. In these tumor cells, the suppression of adrenocorticotropin (ACTH) by the sst5-preferring ligand, BIM-23 268, was correlated with the high expression of sst5 mRNA (35, 36). In these studies, BIM-23 268, as well as the universal SSR ligand, SOM-230, displayed a similar suppression of ACTH release. Moreover, in corticotrope tumors, the presence of D2R has been demonstrated in 80% of the cases (37). However, with the exception of a case report of an ACTH-secreting lung carcinoid (38), no data showed an additive effect of co-treatment with sst5 and dopamine agonists in corticotrope adenomas, and the comparative efficacy of the chimeric molecule, BIM-23A760, has not yet been documented (39).

The new somatostatin/dopamine chimeric molecules also seem effective in inhibiting tumor cell growth, in vitro, to a greater extent than somatostatin and dopamine agonists tested either alone or in combination in human lung cancer cells, expanding the potential indications to more human malignancies (40). Indeed, a heterogeneous expression of SSRs and D2R has recently been described in GEP NET, that co-expresses sst2 and D2R in all cases and sst5 in more than 80% of tumors tested (41). Although the mean level of sst2 and D2R is lower in GEP than in pituitary adenomas, comparative
levels are observed in almost one-half and one-fifth of the tumors respectively. These interesting results should lead to evaluating the possibility of the use of new bi-specific and hybrid somatostatin analogs in the treatment of GEP tumors not only for inhibition of hormone secretion, but also for potential effects on angiogenesis and cell proliferation (41). Moreover, while it should be emphasized that the enhanced in vitro potency of these new compounds might be related to their capacity to induce SSR and DR dimerization, new tumor models might be of help in elucidating this issue as well.

Conclusions

The concept of GPCRs existing as both homo- and hetero-dimers is now largely accepted. Increasing evidence suggest that the dimerization of GPCRs could be important in aspects of receptor biology, particularly in the regulation of their pharmacological and signaling properties, including the recruitment of the G proteins. In addition to the well-established obligatory heterodimerization, which has been shown to be essential for the cell-surface expression and function of a few GPCRs, it is becoming increasingly clear that ‘non-obligatory’ heterodimers displaying pharmacological and/or functional characteristics that differ from those of their constituent monomers can also exist (3).

However, more studies are warranted to establish the general physiological importance of dimerization in different native systems in order to unravel the structural organization of GPCR dimers, as well as to assess whether oligomeric complexes that include more than two receptors may occur. Since these interactions seem regulated in various ways, including via agonist stimulation, the availability of new hybrids, selective and multireceptor ligands will be of great benefit. However, at the functional level, further studies are needed to substantiate the physiological and/or pathological consequences of homo- and hetero-dimerization in vivo, and to determine whether it will be possible to exploit these processes to develop new drugs with unique properties. As SSRs and DRs are excellent GPCR superfamily representatives, pituitary adenomas as well as NET may be excellent models for studying these properties. This may lead to insights that will fundamentally impact the development of future therapeutic approaches.

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