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Suppression of Rat and Human Growth Hormone and Prolactin Secretion by a Novel Somatostatin/Dopaminergic Chimeric Ligand

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As cotreatment of somatostatin (SRIF) and dopamine (DA) agonists reduces GH in acromegaly more effectively than either agonist alone, SRIF and DA receptors (SSTR and DAR) may interact with enhanced functional activity. The selective SSTR2 agonist, BIM-23023 (50% effective dose, 0.42), and the DAR2 agonist, BIM-53097 (50% effective dose, 22.1), dose-dependently inhibited GH secretion in cultured primary rat and human fetal as well as in human pituitary tumor cells derived from GH-secreting adenomas. The combination of individual SSTR2 and DAR2 agonists was additive for suppressing GH secretion in both rat and human pituitary cells. BIM-23A387 is a chimeric compound that contains structural elements of both SRIF and DA in a single molecule and retains potent, selective binding to DAR2 and SSTR2. BIM-23A387 (50% effective dose, 0.16 for SSTR2 and 24.5 for DAR2), displayed similar efficacy in suppressing GH secretion from rat pituitary cells as the combination of the two individual agonists. In contrast, the chimeric molecule was more potent than individual selective analogs in suppressing GH secretion by human fetal pituitary and GH-secreting adenoma cells ($P < 0.05$). Although the DAR2 antagonist, sulpiride, reversed BIM-23A387-induced GH suppression, blockade of SSTR2 by the selective SSTR antagonist, BIM-23454, did not block BIM-23A387-suppressed GH secretion. These results indicate that mechanisms by which the chimeric molecule suppresses pituitary GH secretion may not be mediated by individual SSTR2 or DAR2 signaling, respectively. Functional interaction of the two receptors may explain the clinical observation that more effective GH suppression is achieved when DAR2 and SSTR2 agonists are administered in combination. The SRIF/DA chimeric molecule, BIM-23A387, represents a novel tool for effective drug treatment of acromegaly and for prolactinomas otherwise resistant to dopaminergic therapy. (*J Clin Endocrinol Metab* 88: 5414–5421, 2003)

DOPAMINE (DA) AND somatostatin (SRIF) suppress GH secretion in pituitary tumor cells, and both analogs are used for treating acromegaly (1–3). Combined acromegaly treatment with DA and SRIF agonists reduces both GH and IGF-I levels more effectively than treatment with either agonist alone (4–7). Patients who do not respond well to individual SRIF or DA agonist treatment may in part be more responsive to combined therapy with the two ligands.

The mechanism by which combined SRIF/DA treatment enhances GH suppression is unclear. SRIF agonist treatment may increase the bioavailability of administered DA agonists, thus enhancing efficacy (5). Direct interaction of SRIF and DA in suppressing GH at the cellular level, however, has not been demonstrated. Human DAR receptor 2 (DAR2) and SRIF receptor 5 (SSTR5) were shown to heterooligomerize to enhance functional activity in stably transfected CHO-K1 cells (8). However, ligand-induced heterodimerization between SSTR subtypes and DAR2 has not been demonstrated in pituitary cells.

Normal human pituitary as well human GH-secreting pituitary adenomas express both DA and SRIF receptors (2, 3), and SSTR2 and SSTR5 subtypes both mediate human GH suppression (9, 10). BIM-23A387, a chimeric compound containing structural elements of both SRIF and DA in a single molecule, retains potent, selective binding to both DAR2 and SSTR2 (11).

The purpose of the present study was to examine effects of combined SRIF and DA treatment on GH and prolactin (PRL) secretion at the cellular level and to assess the response when both SRIF and DA activities are combined within the same chimeric molecule.

Materials and Methods

SRIF and DA agonists and antagonists

Peptide compounds were obtained from Biomeasure, Inc. (Milford, MA). BIM-23023 (MW 1032.3) is a potent SSTR2-selective agonist, and BIM-53097 (MW 386.6) is a DAR2 agonist. BIM-23A387 (MW 1372.7) is a chimeric molecule that combines structural elements of both SST and DA within a single molecule, and possesses potent, selective agonist activity for both SSTR2 and DAR2. BIM-23454 is a selective SSTR2 antagonist. Specific binding affinities of these compounds for different human SSTR subtypes and DAR2 were determined by radioligand membrane receptor binding assay as previously described (10) and are summarized in Table 1.

Stock solutions (100 μM) of these compounds were prepared in 0.01 M acetic acid and 0.1% BSA and were stored at −20 C until used. Sulpiride, a DAR2-selective antagonist, was purchased from Sigma-Aldrich Corp. (St. Louis, MO).

Pituitary cell cultures

All human tissue collections were obtained anonymously as approved by the institutional review board for protection of human subjects. Fetal pituitary specimens (gestational age, 18 and 24 wk) were collected anonymously from a third party clinic with informed consent.

Abbreviations: AC, Adenylate cyclase; DA, dopamine; DAR, dopamine receptor; FBS, fetal bovine serum; PRL, prolactin; SRIF, somatostatin; SSTR, somatostatin receptor.

TABLE 1. Human SSTR and DAR subtype specificity

Compound	SSTR					DA D2
	1	2	3	4	5	
Octretrotide	1140	0.56	34.5	7030	7.0	
Lanreotide	2330	0.75	107	2100	5.21	
Cabergoline						3.0[a]
BIM-23023	6616	0.42	86.88	2700	4.18	>1000
BIM-23A387	293	0.16	77.40	ND	1000	24.50
BIM-53097						22.1
BIM-23454	1000	31.6	50.5	301	138.7	

Values are the 50% inhibitory concentration (nanomolar). ND, Not determined.
[a] Ref. 33.

GH- and PRL-secreting pituitary adenoma specimens were obtained at transsphenoidal surgery. Normal rat pituitary tissues were obtained from adult Sprague Dawley rats, as approved by institutional animal use committee. Pituitary cells were prepared as previously described (9, 10). Briefly, pituitary tissue was minced and enzymatically dissociated in DMEM containing 0.35% collagenase, 0.15% hyaluronidase, and 0.3% BSA (all three from Sigma-Aldrich Corp.) at 37 C for 45 min, followed by the addition of fetal bovine serum (FBS) to neutralize enzymes. Pituitary cells collected by centrifugation were cultured in DMEM containing 10% FBS.

GH_3 and MMQ rat pituitary cell lines were purchased from American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 containing 15% horse serum and 2.5% FBS. Pituitary cells were preincubated in serum-containing medium for 48 h, then in serum-free medium containing 0.3% BSA for 3–4 h, followed by treatment for up to an additional 20 h in 48-multiwell tissue culture plates with medium containing 0.3% BSA and the indicated test agents. At the end of each experiment, medium was collected and stored at −20 C until hormone assay.

Hormone assays

Human GH and PRL concentrations in culture medium were measured by RIA and immunoradiometric assay, respectively, with a measurable range of 1–30 ng/ml for human GH and 2.5–200 ng/ml for human PRL (Diagnostic Products Corp., Los Angeles, CA). Rat GH and PRL RIAs were performed using reagents provided by the National Hormone and Peptide Program (Harbor-University of California-Los Angeles Medical Center, Torrance, CA). GH and PRL were iodinated using the Iodogen method (12)

Statistical analysis

Results are presented as the mean ± SEM. Changes in hormone secretion are expressed as a percentage of the mean hormone concentration in vehicle-treated control cultures within the same experiment or as indicated. A *t* test with the Bonferroni correction (for multiple comparisons) was used to determine statistical differences between groups.

Results

Effects of the DA agonist, BIM-53097, on GH secretion

BIM-53097 (0.4–40 nM) reduced GH concentrations in medium from rat pituitary cells cultures to 80 ± 8%, 66 ± 7% ($P < 0.05$), and 64 ± 8% ($P < 0.05$) of the vehicle-treated controls value, respectively (Fig. 1). GH levels in human fetal pituitary cultures were reduced to 97 ± 23%, 51 ± 6%, and 62 ± 6% of the vehicle-treated control level, respectively, by the same range of BIM-53097 concentrations (Fig. 2). BIM-53097 also suppressed GH secretion in cultured GH-secreting pituitary tumor cells to 73 ± 3% ($P < 0.05$), 69 ± 4% ($P < 0.05$), and 68 ± 4% ($P < 0.05$) of the vehicle-treated control level (Fig. 3). These results confirm that DAR2 ligand suppresses GH secretion in GH-secreting adenoma cells as well as in normal human and rat pituitary cells.

Effects of the SSTR2-selective agonist, BIM-23023, on GH suppression

BIM-23023 (0.4–40 nM) dose-dependently inhibited GH secretion from both normal rat and human pituitary cells. GH levels were reduced in rat pituitary cultures to 74 ± 10%, 58 ± 3% ($P < 0.05$), and 38 ± 3% ($P < 0.05$) of the level in vehicle-treated controls, respectively (Fig. 1); GH levels were reduced in human fetal pituitary cultures to 59 ± 5%, 52 ± 8%, and 34 ± 3% ($P < 0.05$) of the vehicle-treated control level, respectively (Fig. 2). In human GH-secreting tumor cells, the same concentrations of BIM-23023 suppressed GH levels to 63 ± 9% ($P < 0.05$), 59 ± 7% ($P < 0.05$), and 72 ± 4% of the vehicle-treated control value, respectively (Fig. 3). These results confirm the responsiveness of rat and human pituitary cells as well as human pituitary GH adenoma cells to SSTR2-selective activation, and further indicate that, in general, SSTR2 activation is more effective in suppressing GH than is DAR2 activation.

Additive effects of BIM-53097 and BIM-23023 on GH suppression

Combined treatment with both SSTR2 and DAR2 agonists was additive for GH suppression in both rat (Fig. 1) and human (Fig. 2) pituitary cultures. This action was observed with lower doses at which lesser suppression occurred with individual SSTR2 and DAR2 agonists. In rat pituitary cultures, GH levels were reduced to 43 ± 4% and 40 ± 4% of vehicle-treated control levels ($P < 0.05$ *vs.* agonist used alone) with combined doses of 0.4 and 4 nM, respectively. In human fetal pituitary cells, GH levels were reduced to 39 ± 11% of vehicle-treated control levels ($P < 0.05$ *vs.* agonist alone) by cotreatment with 0.4 nM of each ligand.

Comparison of GH suppression by combination of SSTR2/DAR2 agonists and the chimeric SST/DA compound, BIM-23A387

The SRIF/DA chimeric molecule, BIM-23A387, was similarly effective in suppressing GH secretion as a cotreatment with the two individual SSTR2 and DAR2 agonists in both normal rat and human fetal pituitary cells (Figs. 1 and 2). In human GH-producing tumor cells, the chimeric molecule (0.4 nM) showed greater GH suppression compared with combined cotreatment with individual SSTR2 and DAR2 agonists (45% *vs.* 63% at 0.4 nM; $P < 0.05$). In these studies the effectiveness of the combination of SSTR2 and DAR2

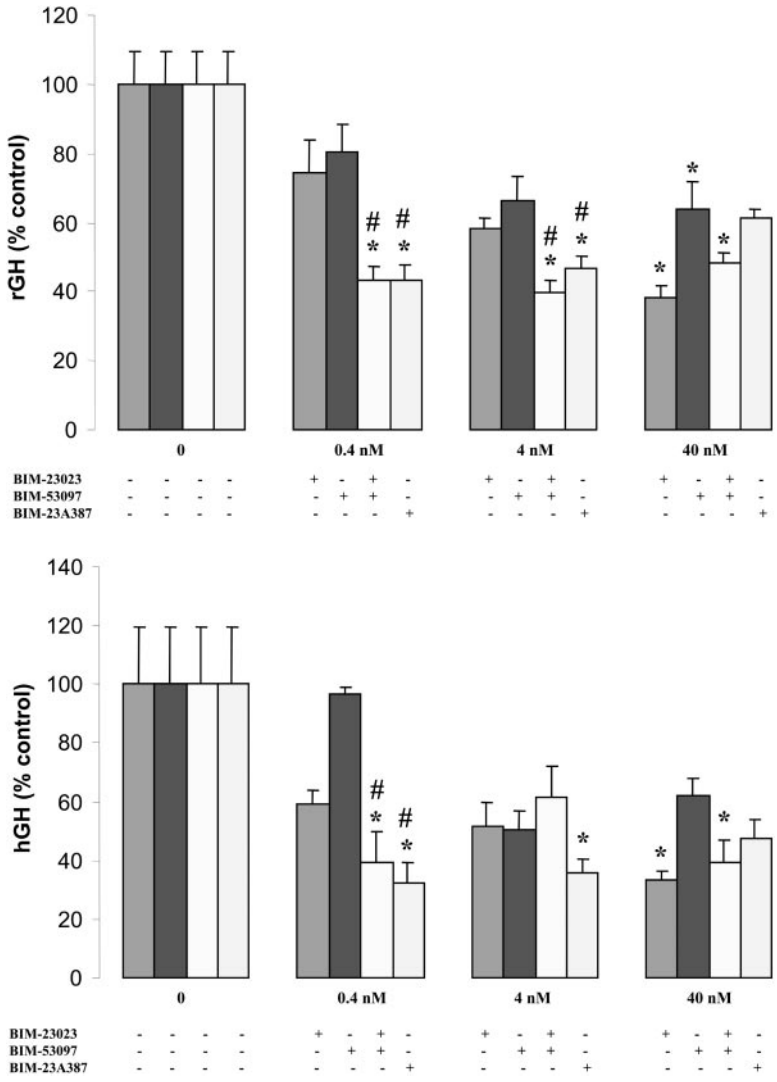

FIG. 1. GH suppression induced by SSTR2 and D2 agonists and a chimeric SSTR2/DAR2 molecule, BIM-23A387, in rat anterior pituitary cells. Each *bar* represents the mean ± SEM hormone concentration after 20 h in 10–20 wells derived from 3 independent experiments. *, $P < 0.05$ *vs.* the zero dose; #, $P < 0.05$ *vs.* agonists used alone.

FIG. 2. GH suppression with SSTR2 and D2 agonists and a chimeric SSTR2/DAR2 molecule, BIM-23A387, in human fetal pituitary cells. Each *bar* represents the mean ± SEM hormone concentration after 20 h in four to six wells in a representative experiment. *, $P < 0.05$ *vs.* the zero dose; #, $P < 0.05$ *vs.* agonists used alone.

agonists or the SRIF/DA chimeric molecule decreased with increasing doses from 0.4 to 40 nM (Figs. 1–3).

Effects of DAR2 and SSTR2 antagonists on BIM-23A387-induced GH suppression

To test the contributions of SSTR2 and DAR2 to the activity of the chimeric molecule, BIM-23A387, the effects of SSTR2- and DAR2-specific antagonists were tested in three experimental models. In rat pituitary cultures (Fig. 4), the GH- and PRL-suppressing activity of the SSTR2 agonist, BIM-23023 (4 nM; GH, 40% of vehicle-treated control; PRL, 66% of vehicle-treated control) was reversed by 200 nM BIM-23454, a selective SSTR2 antagonist (GH, 80% of control; PRL, 83% of control). Similarly, the PRL-suppressing activity of the DAR2 agonist, BIM-53097 (4 nM; 26% of the control; no significant effect on GH secretion was observed at this dose), was reversed (130% of vehicle-treated control) by 200 μM sulpiride, a DAR2 antagonist. Sulpiride also blocked both GH and PRL suppression induced by the combination of BIM-23023 and BIM-53097 [from 46% to 77% of control levels for GH ($P < 0.05$) and from 22% to 71% of control PRL levels ($P < 0.05$)]. Similarly, sulpiride reversed the suppression of both GH and PRL by the SST/DA chimera, BIM-23A387 (from 41% to 82% of control levels for GH and from 24% to 70% of control PRL levels). However, BIM-23454, the selective SSTR2 antagonist, did not reverse BIM-23A387-induced GH or PRL suppression.

Similar results were observed using cultured human fetal pituitary cells. However, in this model, BIM-23A387 induced a greater suppression of GH than BIM-23023 (23% *vs.* 37% of controls, respectively; $P < 0.05$). Treatment with the SSTR2 antagonist, BIM-23454, was again ineffective, whereas treatment with the DAR2 antagonist, sulpiride, fully reversed the suppressive action of the chimeric molecule (Fig. 5).

Using cells cultured from a GH-secreting pituitary tumor (Fig. 6), the SSTR2 agonist, BIM-23023 (4 nM), suppressed GH secretion to 36% of the vehicle-treated control level, and the DAR2 agonist, BIM-53097 (4 nM), suppressed GH to 61% of the control level. In this model the SRIF/DA chimeric molecule, BIM-23A387, suppressed GH secretion to 31% of the control level.

As with the normal pituitary cell cultures, GH suppression caused by BIM-23A387 was reversed by sulpiride, but not by BIM-23454, the SSTR2 antagonist. Cells cultured from two

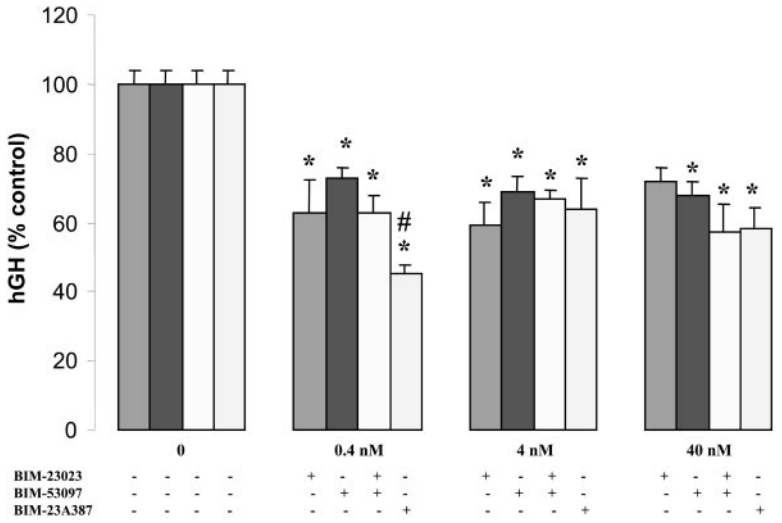

FIG. 3. GH suppression with SSTR2 and D2 agonists and a chimeric SSTR2/DAR2 molecule, BIM-23A387, in cells derived from a human GH-secreting pituitary adenoma cells. Each *bar* represents the mean ± SEM hormone concentration after 20 h in four to six wells. *, $P < 0.05$ *vs.* the zero dose; #, $P < 0.05$ *vs.* agonists used alone.

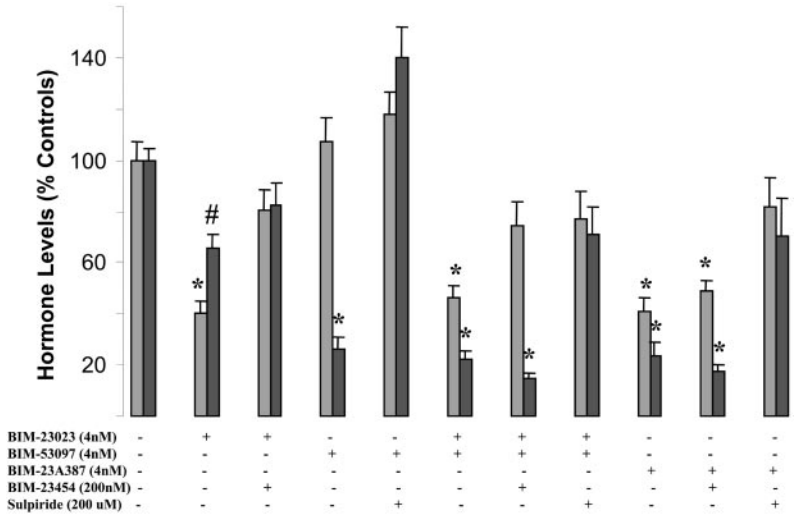

FIG. 4. Effects of SSTR2 and D2 agonists and antagonists on GH and PRL secretion in primary rat pituitary cell cultures. Medium GH (▨) and PRL (■) were measured after 20 h. Each *bar* represents the mean ± SEM hormone secretion in 12–20 wells from 3 independent experiments. *, $P < 0.05$ *vs.* control (#, *t* test only).

additional GH-secreting pituitary tumors and one PRL-secreting pituitary tumor showed similar results. Collectively, these results indicate that, as expected, the SSTR2 antagonist selectively reverses SSTR2 agonist-induced hormone suppression, and the DAR2 antagonist selectively reverses DAR2 agonist-induced hormone suppression. However, unexpectedly, when the DAR2 component of BIM-23A387 activity was blocked by sulpiride, the SSTR2 component of BIM-23A387-induced GH suppression was also blocked. When the SSTR2 component of BIM-23A387 was blocked by BIM-23454, the DAR2 component of BIM-23A387 remained more effective in suppressing GH than the individual DAR2 agonist, BIM-53097. These results suggest that GH suppression by BIM-23A387 is not mediated through either individual SSTR2 or DAR2 mechanisms, but via a functional interaction between the two receptors, in which DAR2 appears to be the dominant element.

Supporting experiments in GH_3 and MMQ cell lines

To substantiate the hypothesis that the activity of BIM-23A387 requires a functional interaction between SSTR2 and DAR2, the rat pituitary adenoma cell line GH_3, which does not express functional DAR2 (13), was used. Neither the DAR2 agonist, BIM-53097, nor the SRIF/DA chimeric molecule, BIM-23A387, suppressed either GH or PRL secretion in GH_3 cells (Fig. 7). Conversely, in MMQ cells, a rat lactotroph cell line that expresses both STTR2 and DAR2 (13), BIM-23A387 dose-dependently inhibited PRL secretion, as shown in Fig. 8. These results provide further evidence that DAR2 is required for BIM-23A387 activity in suppressing both GH and PRL secretion.

Comparison of PRL suppressive activity among SST and DA agonists

To compare the efficacy of a molecule exhibiting both SSTR2 and DAR2 activity with respect to PRL secretion, the chimeric molecule, BIM-23A387, was compared with selective agonists for SSTR2 and DAR2 for their ability to suppress PRL secretion from cultured cells derived from a human prolactinoma.

As shown in Fig. 9, the SSTR2 preferential agonist, octreotide (10 nM), suppressed PRL secretion from prolactinoma

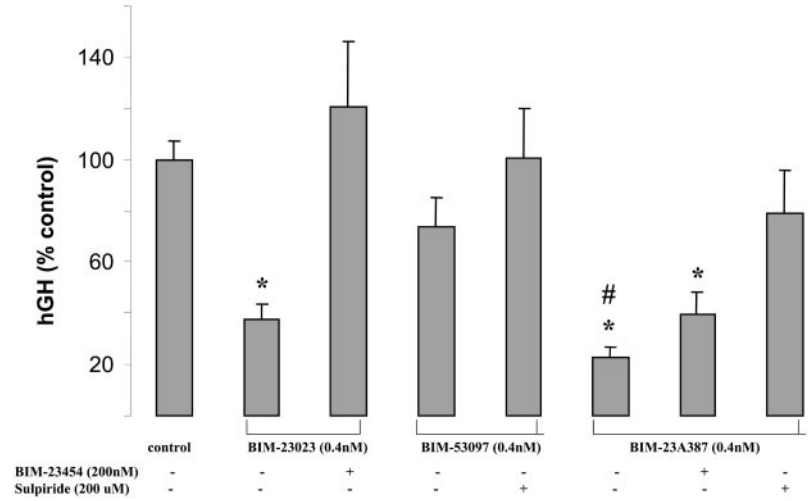

FIG. 5. Effects of SSTR2 and D2 agonists and antagonists on GH and PRL secretion in human fetal pituitary cell cultures. Each *bar* represents hormone secretion after 20 h in eight wells from two independent experiments. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* BIM-23023 or BIM-53097 used alone.

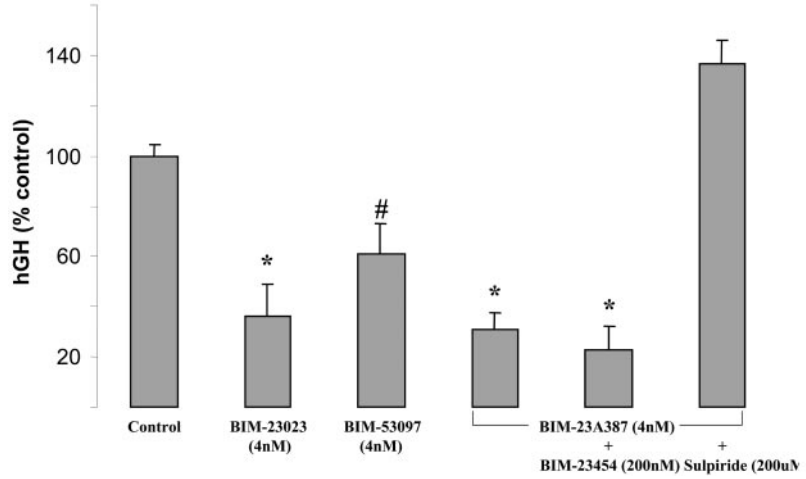

FIG. 6. Effects of SSTR2 and D2 agonists and antagonists on GH secretion in human GH-secreting adenoma cells. Each *bar* represents the mean ± SEM hormone secretion after 20 h in eight wells from a representative experiment performed three times. * and #, $P < 0.05$ *vs.* control.

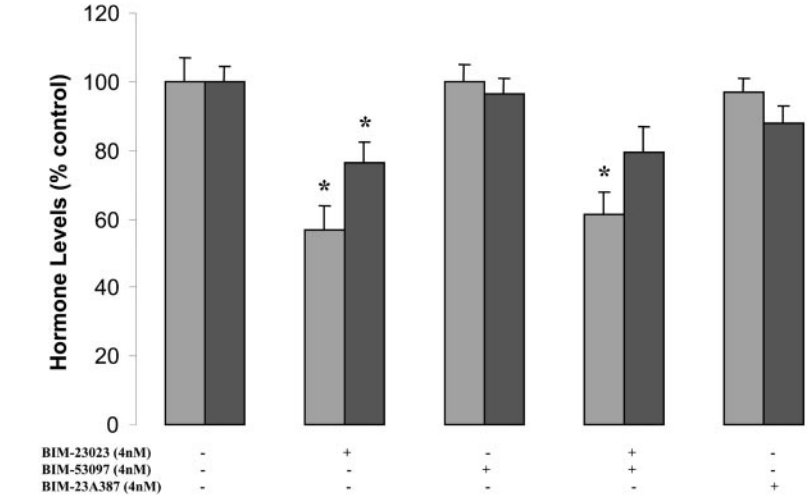

FIG. 7. Effects of SSTR2 and D2 agonists on GH and PRL secretion in rat GH_3 cells. ▨, GH levels; ■, PRL levels. Each *bar* represents the mean ± SEM hormone secretion after 20 h in 8–20 wells from three separate experiments. *, $P < 0.05$ *vs.* controls.

cells to 77%, 61% ($P < 0.05$), and 60% ($P < 0.05$) of control levels after 3, 6, and 20 h of exposure, respectively. The DA agonist, bromocriptine (10 nM), suppressed PRL to 74%, 46% ($P < 0.05$), and 39% ($P < 0.05$) of control levels after 3, 6, and 20 h, respectively. Similar results were observed with the DAR2-selective agonist, BIM-53097 [72%, 59% ($P < 0.05$), and 43% ($P < 0.05$) of control levels at 3, 6, and 20 h, respectively]. BIM-23A387, the chimeric SRIF/DA molecule, suppressed PRL by 62% ($P < 0.05$), 50% ($P < 0.05$), and 30% ($P < 0.05$) of control levels at 3, 6, and 20 h, respectively, thus demonstrating greater efficacy for PRL suppression in prolactinoma cells.

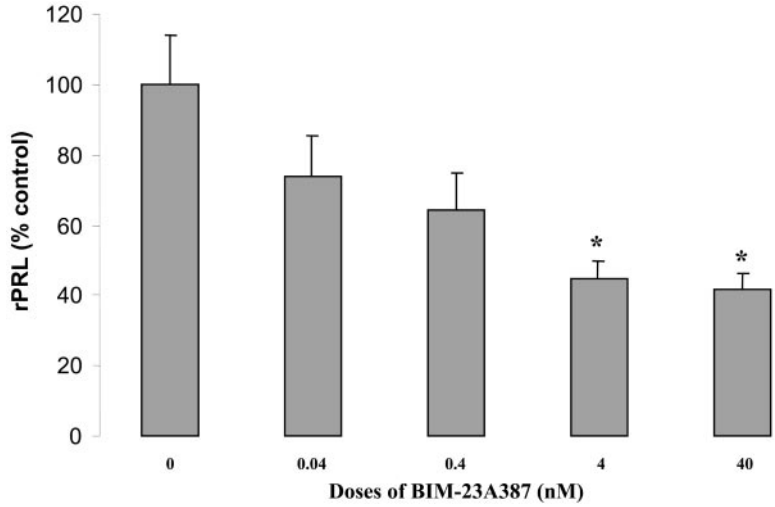

FIG. 8. BIM-23A387 dose-dependently suppresses PRL secretion from rat MMQ cells. Each *bar* represents the mean ± SEM hormone secretion after 20 h in four wells in a single representative experiment performed three times. *, $P < 0.05$ *vs.* the zero dose.

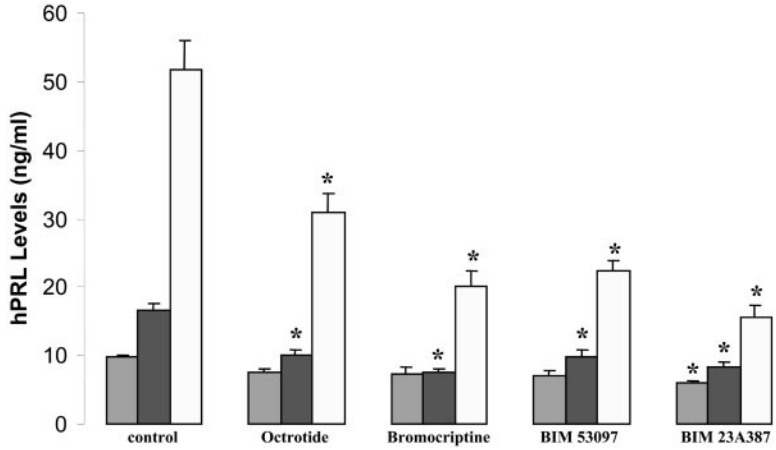

FIG. 9. SRIF and D2 agonists (10 nM) suppress PRL secretion from cultured human prolactinoma cells. Cells were treated for 3 h (▨), 6 h (■), and 20 h (□). Each *bar* represents the mean ± SEM of four wells. *, $P < 0.05$ *vs.* controls.

Discussion

BIM-23A387, a novel compound that combines structural elements of SRIF and DA ligand activity within a single molecule, is shown here to possess selective agonist activity at both SSTR2 and DAR2 receptors. The results also demonstrate a functional interaction between SSTR2 and DAR2 for regulating GH and PRL secretion in pituitary cells.

We confirmed previously reported *in vivo* observations that SRIF and its agonists are more effective than DA in suppressing GH secretion, and that DA and its agonists are more effective than SST in suppressing PRL secretion (2, 3, 13–15). The results show that cotreatment with lower doses of SSTR2 and DAR2 agonists produce enhanced GH suppression, greater than that produced by activation of either receptor alone. These results support, at the cellular level, the clinical observation that SRIF and DA analogs more effectively suppress GH in subjects with acromegaly when used in combination (4–7). The SRIF/DA chimeric molecule, BIM-23A387, showed comparable or greater effectiveness in suppressing GH secretion compared with individual SSTR2 and DAR2 agonists added in combination, consistent with data recently reported (11).

The DAR2 selective antagonist, sulpiride, blocked activity of the DAR2 dopaminergic component of the chimeric molecule, as well as the SSTR2 component. However, blockade of the SSTR2 receptor by a SSTR2 selective antagonist, BIM-23454, produced no effect. In comparing results using the MMQ and GH3 rat pituitary cell lines, which do or do not express functional DAR2, respectively (13), the presence of endogenous DAR2 was shown to be a requirement for BIM-23A387 suppression of both GH and PRL. Collectively, these results suggest that GH suppression by the chimeric SRIF/DA molecule, BIM-23A387, is not mediated through either individual SSTR2 or DAR2 receptors, but requires a functional interaction between the two receptors in which DAR2 appears to have a dominant role. This functional interaction may explain enhanced GH suppression when DAR2 and SSTR2 agonists are used in combination therapeutically.

In contrast, a recent report indicated that blockade of either the DAR2 or the SSTR2 in primary cultures of human GH-secreting adenoma cells caused only a partial inhibition of the GH suppression induced by BIM-23A387 (11). Combined blockade of both DAR2 and SSTR2 was required to fully suppress BIM-23A387 action. The reason for the difference between these results and those of the present study is unclear. One possibility may be that differences in culture conditions resulted in different expression/interaction of the two receptor subtypes. Alternatively, the relatively elevated PRL levels (35 ng/ml) (11) may reflect a different cell type.

Another possible reason is that we used maximal doses (0.4 and 4 nM), whereas Saveanu *et al.* (11) used a 50% effective dose (1 pM). At lower doses, BIM-23A387 ligand-induced heterodimerization of SSTR2 and DAR2 may not be sufficient to saturate the chimeric molecule, so that free BIM-23A387 is available for banding to SSTR2 or DAR2 alone. Regardless of the specific differences, the results from both studies support the concept of a functional interaction between SSTR2 and DAR2 in mediating the actions of the chimeric molecule, BIM-23A387.

Functional interaction between SSTR subtypes was also observed in our previous study (16), in which a biselective analog for SSTR2/SSTR5 appeared to be more effective than a SSTR subtype-specific analog alone in suppressing human fetal pituitary GH secretion with similar inhibitory action by BIM-23A387.

SSTR2 and DAR2 receptors are abundantly colocalized in pituitary cells and are both G protein-coupled receptors that interact with multiple intracellular pathways (13, 15). However, signal transduction mechanisms by which both DA and SRIF suppress GH and PRL have not been fully elucidated (13, 15). DAR and SSTR transduction pathways may be coupled through different G proteins, which may explain the different inhibitory activities of SRIF and DA for GH and PRL. For example, steroids modulate the sensitivity of the pituitary cell to SST by increasing the number of specific binding sites, whereas cellular sensitivity to DA is modulated by altered DAR2 transduction mechanisms (17). Both SSTR2 and DAR are negatively coupled to adenylate cyclase (AC), such that activation of either receptor inhibits AC activity and reduces cAMP, effects that can be abolished by pertussis toxin and increased by GTP (18). Higher GTP doses were required for DA inhibition of AC activity than for SRIF in membrane preparations derived from pituitary cells pretreated with pertussis toxin, suggesting that different G proteins are involved in DAR and SSTR coupling to AC (18). Both DA and SST also suppress cytosolic free calcium levels in pure GH-secreting adenoma cells, whereas in these same cells, SRIF, but not DA, suppresses AC activity (18). However, in tumors predominantly composed of mammosomatotrophs, DA does inhibit AC activity (19), suggesting a differential signal transduction mechanism for DA in different GH-secreting adenoma subtypes and supporting the observation in several clinical studies that DA agonists are primarily effective in GH-secreting tumors that cosecrete PRL (20, 21). Interestingly, the activities of both SRIF and DA have been shown to ultimately converge on similar K^+ channels in human GH-secreting cells (22).

Recent studies provide evidence that G protein-coupled receptors may pair up with related (even rather distantly related) relatives to form heterodimeric units with distinct properties (23). The observed functional interaction between SSTR2 and DAR2 may reflect the formation of a heterodimer between the two receptors, as has been suggested from recent studies of the SSTR5 and DAR2 receptors (8).

Dimerization between DAR2 and SSTR2 allows either DA or SST to function through either or both DAR2 and SSTR2 signal transduction pathways. This phenomenon has been demonstrated in studies using cotransfection of a SSTR5 signaling domain mutant and either a full-length binding domain mutant DAR2 (8) or mutant SSTR5 and SSTR1 (24). A recent study showed that ligand-induced hetrooligomerization of DAR2 and SSTR2 occurs not only in a transfected cell line, but also in central neurons, as assessed by fluorescence resonance energy transfer (25). In the present study a DAR2 antagonist abolished the SSTR2-active component of BIM-23A387 to inhibit GH secretion, whereas an SSTR2 antagonist was ineffective. This observation is consistent with previous results (8), in which sulpiride blocked the ability of SRIF to inhibit forskolin-stimulated cAMP in cells cotransfected with DAR2 and signaling domain mutant SSTR5 receptors (8). This phenomenon may be explained by SSTR2 and DAR2 antagonists possessing different conformational states for occupying the putative heterodimeric receptor.

The structure of the DAR2/SSTR2 heterodimer may differ from that of the individual DAR2 or SSTR2, and the heterodimer binding domain may prefer DAR2-selective ligands, whereas intracellular signaling may occur mainly through original SSTR2 signaling pathways.

Higher doses (0.4–40 nM) of BIM-23A387 were used in this dose-response study than those employed by the Jaquet group (from 1 pM to 10 nM) (11). At higher doses of BIM-23A387, a dose-dependent PRL inhibition was observed in MMQ cells, possibly reflecting higher levels of SSTR2 and DAR2 expression. However, the SRIF/DA chimeric molecule, BIM-23A387, showed higher efficacy in suppressing GH secretion from primary pituitary cell cultures at a lower dose (0.4 nM), than that at higher doses (4 and 40 nM). If the SSTR2 and DAR2 receptors indeed form heterodimers in response to BIM-23A387, the observed reduced efficacy at higher doses may be due to a concentration-dependent dimer formation. In the absence of ligand, DAR2 and SSTR2 may exist separately and may dimerize with low concentrations of BIM-23A387(0.4 nM), whereas at higher doses (40 nM), BIM-23A387 binds all available DAR2 or SSTR2 monomeric receptors, so that there are no available DAR2 and SSTR2 monomers to form heterodimers, as has been described for GH and erythropoietin receptor dimerization (26).

The sequences of SRIF subtypes from different species are highly conserved with 81–97% identity, and human and rodent forms of SSTR1, -2, -3, and -4 have similar ligand properties (27). Species, sex, and physiological differences in GH responses to DA and its agonists differ (12–14), as do altered responses due to different neuroendocrine GH control mechanisms. In the *in vitro* studies shown here, DA-induced GH suppression did not differ appreciably in rat and human cells or in normal pituitary and pituitary adenoma cells, suggesting that the functional interaction of SSTR2 and DAR2 occurs in various species and under different physiological or pathological conditions. The differences between normal pituitary and adenoma cells, as suggested by the results illustrated in Figs. 2 and 3, may reflect differences in DAR2 or SSTR2 expression.

In addition to the direct interaction of pituitary SRIF and DA receptors, central pathways may also be involved in a functional linkage between DAR2 and SSTR2 *in vivo*. Dopamine releases GHRH and SRIF from the hypothalamus, and the final GH response to dopamine is dependent on the endogenous DA tone (15). Induction of dopaminergic blockade in 10 normal human subjects increased GHRH-induced

GH secretion and caused a loss of the relationship between pre-GHRH plasma GH values and GHRH-elicited GH peaks, suggesting that central DA may stimulate SRIF secretion (28). DA-induced SRIF release has also been observed in the rat (29). DA and DA agonists also increase the number and activity of SSTRs in rat striatum (30, 31). Conversely, SRIF and SSTR2 agonists stimulate rat DA release (32).

BIM-23A387, a chimeric SRIF/DA agonist that is shown here to be a potent GH inhibitor at the cellular level, may also exhibit additional GH-suppressive functions *in vivo* through possible central interaction between SSTR2 and DAR2. Thus, BIM-23A387 represents a new pharmacological concept that may prove useful and convenient in the treatment of patients with acromegaly and hyperprolactinemia. Patients whose adenoma tissues express both SSTR2 and DAR2 may be especially suitable to benefit from BIM-23A387 at lower doses.

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