0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 1
Printed in U.S.A.

BIM-23244, a Somatostatin Receptor Subtype 2- and 5-Selective Analog with Enhanced Efficacy in Suppressing Growth Hormone (GH) from Octreotide-Resistant Human GH-Secreting Adenomas*

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ABSTRACT

Although both somatostatin receptor subtype 2 (SSTR2) and SSTR5 messenger ribonucleic acid (mRNA) are consistently expressed in GH-secreting adenomas, SSTR2 has been believed to be the key modulator of somatostatin-mediated inhibition of GH release. The somatostatin agonists currently in clinical use, octreotide and lanreotide, are directed mainly to SSTR2 (IC_{50} 12- to 18-fold higher than for SSTR5). Recently, however, it was demonstrated that an SSTR5 preferential agonist, BIM-23268, not only suppressed PRL release from prolactinomas and mixed GH-PRL adenomas, but also inhibited GH release in about half of GH adenomas. In addition, the SSTR5-preferring analog showed a slight additive effect when used in combination with SSTR2 preferential drugs at submaximal concentrations in octreotide partially sensitive adenomas. In the present study we quantified SSTR2 and SSTR5 mRNA expression and the GH-suppressive effects of somatostatin-14; octreotide; a SSTR2-preferential compound, BIM-23197; a SSTR5-preferential compound, BIM-23268; and a new SSTR2- and SSTR5-bispecific compound, BIM-23244, in GH-secreting tumors classified as either full responders to octreotide (n = 5) or partially sensitive to octreotide (n = 5). The octreotide-sensitive GH secretory adenomas presented with a high level of both SSTR2 and SSTR5 mRNA expression [222 ± 61 and 327 ± 136 pg/pg glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively]. In these tumors the suppression of GH release was similarly achieved at picomolar ranges by octreotide, BIM-23197, and BIM-23244 (EC_{50} = 25 ± 15, 3 ± 2, and 3 ± 3 pmol/L, respectively). The compounds preferential for only SSTR5 were unable to inhibit GH release in such tumors. Among the octreotide partially responsive tumors, SSTR2 mRNA expression was 9-fold lower than in the octreotide-sensitive tumors (25 ± 12 *vs.* 222 ± 61 pg/pg GAPDH; $P < 0.015$), whereas SSTR5 mRNA expression was approximately 7-fold higher than in the octreotide-sensitive tumors (2271 ± 1197 pg/pg GAPDH). In these octreotide partially responsive tumors, the SSTR5-preferential compound, BIM-23268, and the SSTR2- and SSTR5-bispecific compound, BIM-23244, were quite effective in suppressing GH secretion (EC_{50} = 25 ± 13 and 50 ± 31 pmol/L, respectively). Similarly, BIM-23244, was able to suppress by 51 ± 5% PRL release from five mixed GH- and PRL-secreting adenomas. These data indicate that due to heterogeneous expression of SSTR2 and SSTR5 receptor subtypes, in GH-secreting tumors, a bispecific analog, such as BIM-23244, that can activate both receptors could achieve better control of GH hypersecretion in a larger number of acromegalic patients. (*J Clin Endocrinol Metab* **86:** 140–145, 2001)

THE SOMATOSTATIN (SRIF) agonists, octreotide and lanreotide, have been widely used in the treatment of acromegalic patients. Worldwide experience with long-term treatments using these SRIF analogs has resulted in normalized GH and insulin-like growth factor I (IGF-I) levels in about 50% of patients (1–3). To explain the lack of full efficacy of such drugs in half of the acromegalic patients, a loss of SRIF receptor binding capacity was proposed in two different studies (4, 5). In fact, decreased efficacy of SRIF agonists associated with a significant decrease in SRIF receptors represents in these series less than 20% of the cases. Somatostatin exerts its biological effects via five distinct high affinity receptor (SSTR) subtypes that belong to the family of G protein-coupled receptors (6). Recent studies using subtype-selective SRIF analogs demonstrated the involvement of both SSTR2 and SSTR5 receptor subtypes in regulating GH secretion from human pituitary adenomas (7, 8). As octreotide and lanreotide both have 12- to 18-fold lower binding affinities for SSTR5 than for SSTR2 (9), it is possible that their partial efficacy on the control of GH secretion in some acromegalic patients could be the consequence of their lower affinity for the SSTR5 subtype.

In the present study we used *in vivo* data to select a series of tumors from acromegalic patients considered either full octreotide responders or partial responders. In these cases a portion of the tumor tissue obtained after transsphenoidal surgery was analyzed in terms of SSTR2 and SSTR5 mes-

Received July 18, 2000. Revision received September 13, 2000. Accepted September 14, 2000.

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* This work was supported in part by a grant from Biomeasure, Inc., and a grant from Ligue Nationale contre le Cancer.

senger ribonucleic acid (mRNA) expression. The remainder of the tumor tissue was used for cell culture experiments in which the GH- and PRL-suppressive effects of SRIF-14 and of different SRIF analogs that are selective for the SSTR2-, SSTR5-, and SSTR2- plus -5 subtypes were analyzed. The main objectives in the present work were to characterize the quantitative mRNA expression of GH tumors from octreotide poorly responders and to extend in this group the preliminary observations (7, 8) showing a better suppressive effect on GH suppression of somatostatin analogs preferential for both SSTR2 and SSTR5 subtypes.

Subjects and Methods

Patients

The present study was undertaken after obtaining informed consent from each patient. Ten acromegalic patients (seven women and three men), aged 26–62 yr, presenting with macroadenoma were studied. Their endocrine status and the neuroradiological characterization of the pituitary adenomas were documented before treatment. Basal GH levels were the mean of three random samples obtained between 0800–0900 h. The basal IGF-I value was evaluated under fasting conditions between 0800–0900 h. Magnetic resonance imaging revealed adenomas with a maximal 11- to 42-mm diameter. SRIF agonist sensitivity was assessed by an acute test using a single 200-μg injection of octreotide (Sandostatin, Novartis, Basel, Switzerland). Sensitivity to somatostatin analogs was expressed as the percent decrease in GH from the basal value to the mean GH values 2–6 h after octreotide injection. According to the test results, five patients were considered full octreotide responders (mean GH suppression, 79 $\pm$ 7%), whereas the other five cases were considered partial octreotide responders (mean GH suppression, 33 $\pm$ 6%). All patients underwent transsphenoidal surgery. The clinical endocrine and tumoral status of each patient is summarized in Table 1.

Hormone assays

GH and PRL were measured using commercial immunoradiometric kits (Immunotech, Marseilles, France). Normal GH values ranged from 0.2–2.4 μg/L; normal PRL values ranged from 1–24 μg/L in women and from 1–17 μg/L in men. After an ethanol-acid extraction, the plasma IGF-I assay was performed using the IGF-I RIA kit from Nichols Institute Diagnostics (San Juan Capistrano, CA). The normal ranges, according to sex and age, were established by our laboratory.

Detection of SSTRs

Total RNA was extracted from 30–60 mg tissue from each tumor using the SV total RNA isolation system (Promega Corp., Lyon, France). The RNA samples were subsequently treated with 30 U ribonuclease-free deoxyribonuclease I (Roche, Mannheim, Germany). Total RNA was reverse transcribed into complementary DNA using 1 μg hexamers (Pharmacia Biotech, Orsay, France) and Moloney murine leukemia virus reverse transcriptase, as described by the manufacturer.

The 5′-exonuclease (Taq Man) assay, which produces a direct proportional readout for the progression of PCR reactions, was used to quantify the SSTRs mRNA (10). The details of reaction conditions, the primers used, and the quantification calculation for SSTR2 and SSTR5 mRNA were described previously (8). The results were expressed as picograms of SSTR per pg glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Cell culture studies

A portion of each tumor obtained at surgery was dissociated by mechanical and enzymatic methods. Depending on the tumor, 4–90 $\times$ 10^6 isolated cells were obtained. Tumor cells were initially cultured in DMEM supplemented with 10% FCS for 3 days. On day 3, the cells were washed and plated in multiwell culture dishes (Costar 3524, Brumath, France) coated with extracellular matrix from bovine endothelial corneal cells as previously described (11) at a density of 2×10^4 cells/well. When they were attached to the matrix on days 5–8, depending on the culture, the medium was removed and replaced with DMEM supplemented with 2% FCS, antibiotics, transferrin, and selenium as previously described (11). The effects of various doses of SRIF-14; octreotide; a SSTR2-preferential compound, BIM-23197; a SSTR5-preferential compound BIM-23268; and the SSTR2- and SSTR5-selective compound, BIM-23244, on the inhibition of GH and PRL release were measured over an 8-h period between days 5–8 of culture. Each drug concentration was tested in quadruplicate.

Products

SRIF-14 was purchased from Sigma (Saint-Quentin Fallavier, France). Octreotide was supplied by Novartis (Basel, Switzerland). The BIM compounds were provided by Biomeasure, Inc. (Milford, MA). The human SSTR affinities (IC_{50}; nanomoles per L) of each compound, determined by radioligand receptor binding assays to membranes from transfected CHO-K1 cells expressing the different human SSTR subtypes, are summarized in Table 2. The native SRIF and SRIF analogs were dissolved in 0.01 mol/L acetic acid containing 0.1% purified serum albumin (Life Technologies, Inc., Cergy-Pontoise, France). The drugs were stored at -80 C as 10^{-3} mol/L solutions. For each experiment, fresh working solutions were prepared from a new aliquot.

Statistics

The results are presented as the mean $\pm$ SEM. Statistical significance between two unpaired groups was determined by the Mann-Whitney

TABLE 1. Clinical characterization and RT-PCR quantification of SSTR2 and SSTR5 mRNAs in acromegalic patients

Case no.	Sex	Age (yr)	Tumor size (mm)[a]	GH (μg/L)		PRL (μg/L)	IGF-I (μg/L)	SSTR subtype[b]	
				Basal	Under octreotide[c]			SSTR2	SSTR5
A1	M	53	11	11 → 1 (91)		11	1171	371	555
A2	F	39	15	22 → 4 (82)		17	885	366	744
A3	F	44	13	109 → 26 (76)		12	881	153	129
A4	M	32	15	141 → 36 (75)		2	1010	127	130
A5	F	40	39	47 → 12 (75)		17	1008	93	75
A6	F	62	22	5 → 3 (40)		18	63	ND	ND
A7	M	53	25	26 → 15.8 (39)		19	1100	19	262
A8	F	39	18	13 → 8.2 (37)		17	1187	20	4866
A9	F	26	38	141 → 100 (29)		63	740	59	3745
A10	F	30	42	195 → 151 (23)		30	849	2	210

[a] Maximal tumor diameter was evaluated by MRI.
[b] SSTR2 and SSTR5 mRNA expression in each adenoma is shown. Results are expressed as picograms of SSTR mRNA per pg GAPDH mRNA.
[c] Mean GH values were determined 2-6 h after acute octreotide challenge (200 μg, sc). Percent inhibition *vs.* GH basal value is indicated in *parentheses.*
ND, Not done. Cases 1 and 3 were presented in a previous study (8).

test. To measure the strength of association between the pairs of variables without specifying dependencies, Spearman order correlations were used. $P < 0.05$ was considered significant for all tests.

Results

Correlation between octreotide sensitivity and SSTR2 and SSTR5 subtype mRNA expression in acromegaly

The degree of GH inhibition by SRIF-14 *in vitro* and the levels of SSTR2 mRNA expression have been previously shown to be highly correlated (8). In the present series the degree of GH inhibition in patients, as evaluated by acute octreotide test, was also highly correlated to the level of SSTR2 mRNA expression ($P < 0.009$; Table 1).

In five adenomas (A1–A5) highly sensitive to octreotide, the mean SSTR2 expression was 222 ± 61 pg/pg GAPDH. When the same analysis was made in four of five (A7–A10) adenomas from partial octreotide responders, the mean SSTR2 mRNA expression was much lower (25 ± 12 pg/pg GAPDH). As shown in Fig. 1, the five adenomas from octreotide-responsive patients expressed SSTR5 mRNA at an equivalent level (SSTR2/SSTR5 mRNA ratio, 0.9 ± 0.3). In contrast, adenomas from the four partial octreotide responders with low SSTR2 mRNA expression expressed high levels of SSTR5 mRNA (2271 ± 1197 pg/pg GAPDH). Thus, these data establish two patterns of mRNA expression in the GH-secreting tumors. The octreotide-sensitive adenomas equally express both SSTR2 and SSTR5 mRNA, whereas in the adenomas that were poorly responsive to octreotide, the loss of SSTR2 mRNA contrasted with a 30-fold higher expression of SSTR5 *vs.* SSTR2 mRNA.

TABLE 2. Human somatostatin receptor subtype specificity of SRIF-14 and somatostatin analogs

	SSTR binding affinity (IC_{50}, nmol/L)				
Compound	hSSTR1	hSSTR2	hSSTR3	hSSTR4	hSSTR5
Somatostatin-14	1.95	0.25	1.2	1.7	1.4
Octreotide	1140	0.6	34.5	7030	7
Lanreotide	2129	0.7	98	1826	12.7
BIM-23197	6016	0.19	26.8	3897	9.8
BIM-23268	12	28	5.5	36	0.42
BIM-23244	1020	0.29	133	>1000	0.67

Data from radioligand binding assays to membranes from transfected CHO-K1 cell expressing the different human SSTR (hSSTR) subtypes. Values are from Biomeasure, Inc. (Culler, M. D., personal communication) and from Shimon *et al.* (9).

Effects of SSTR2- and SSTR5-preferential agonists on GH secretion (Fig. 2)

In this series of experiments, the dose-response inhibition of GH release was examined with 10^{-13}–10^{-8} mol/L concentrations of SRIF-14; the SSTR2-preferential compound, BIM-23197; and the SSTR5 preferential compound, BIM-23268. Among the 10 adenoma cell cultures, 2 patterns of responses to SSTR2- and SSTR5-preferential analogs were observed. In cultures from the 5 octreotide-sensitive tumors (A1–A5), the SSTR2-preferential compound, BIM-23197, produced a maximal 41 ± 7% mean GH suppression at a 0.1 nmol/L concentration, with an EC_{50} of 3 ± 2 pmol/L. A similar dose-response inhibition of GH release was obtained with SRIF-14. In contrast, the SSTR5-preferential compound, BIM-23268, produced a maximal inhibition of GH release only at 10 nmol/L (EC_{50} = 800 ± 350 pmol/L). This discrepancy between the results obtained with BIM-23197 and BIM-23268 can be explained on the basis of the binding affinities of BIM-23268, which is preferential for SSTR5, but at high con-

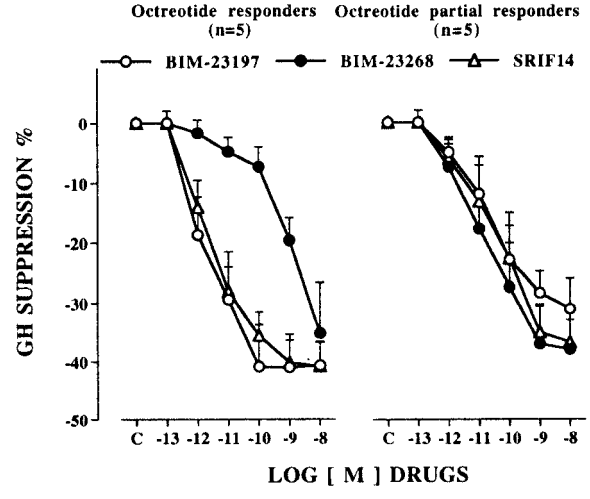

FIG. 2. Mean dose-response GH suppression curves obtained with SRIF-14; the SSTR2- preferential compound, BIM-23197; and the SSTR5-preferential compound, BIM-23268, in cell cultures from 10 GH adenomas. Results are expressed as the mean ± SEM percent GH suppression *vs.* that with medium alone (c, control). The subclasses of octreotide responders (n = 5) or partial responders (n = 5) were defined by *in vivo* octreotide sensitivity, as shown in Table 1, for each tumor.

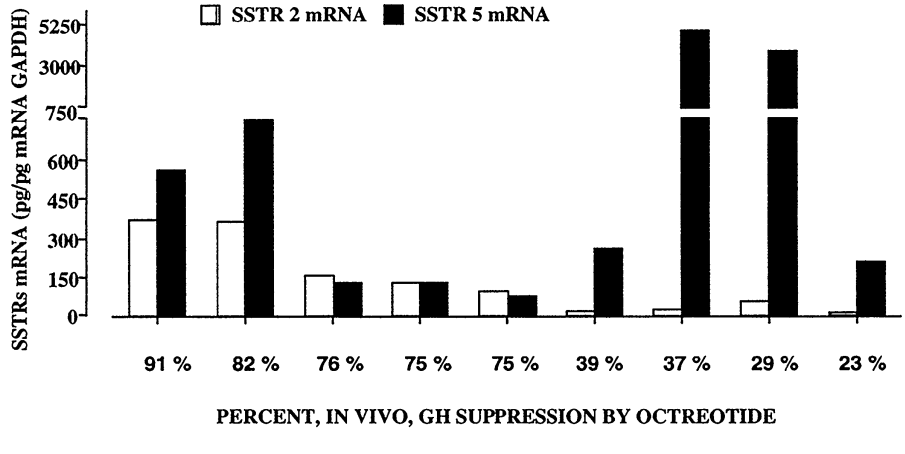

FIG. 1. Quantitative RT-PCR expression of SSTR2 and SSTR5 mRNAs in 9 of 10 GH-secreting adenomas. The 9 analyses were ranked according to *in vivo* sensitivity to octreotide (see Table 1). Results are expressed as picograms per pg GAPDH. The percent GH suppression by octreotide challenge is defined in *Materials and Methods*.

centrations behaves as a weak SSTR2 agonist. Thus, in the tumor cells from full octreotide responders, the GH-suppressive effect of somatostatin was mediated through only the SSTR2 subtype. In the second class of GH-secreting tumors that were partially responsive to octreotide (A6–A10), maximal GH suppression was equally achieved by SRIF-14 and the SSTR5-preferential agonist, BIM-23268. In these 5 adenoma cell cultures, BIM-23197 was slightly less potent than BIM-23268 (maximal GH suppression, 31 ± 5% and 38 ± 7%, respectively). The EC_{50} values achieved with BIM-23268 and BIM-23197 were 25 ± 13 and 47 ± 18 pmol/L, respectively. These data indicate that in tumor cells that are partially responsive to octreotide, the GH-suppressive effect of SRIF is mediated through both the SSTR5 and SSTR2 subtypes.

BIM-23244 vs. octreotide in the octreotide-sensitive and octreotide partially sensitive tumors (Fig. 3)

In the five octreotide-sensitive tumors in which the GH-suppressive effect of SRIF was mediated through the SSTR2 subtype, the effects of the SSTR2- plus SSTR5-selective analog, BIM-23244, and octreotide on GH secretion were examined using 10^{-3}–10^{-8} mol/L of each compound. The dose-response inhibition curves of GH release induced by BIM-23244 and octreotide were parallel (EC_{50} = 3 ± 3 and 55 ± 15 pmol/L, respectively). At nanomolar concentrations, the mean maximal GH suppressions induced by BIM-23244 and octreotide were 44 ± 5% and 36 ± 7%, respectively. These results show that when the GH-suppressive effect is mediated through the SSTR2 subtype, native SRIF and BIM-23244 are similarly efficacious in suppressing GH secretion. As expected from the binding affinities for SSTR2 (Table 2), BIM-23244 was slightly more potent than octreotide.

The same dose-response inhibitions of GH release by BIM-23244 and octreotide were examined in adenoma cell cultures from the five (A6–A10) octreotide partially responsive tumors. The dose-related pattern of GH inhibition induced by octreotide (EC_{50} = 200 ± 145 pmol/L) was markedly distinct from that induced by BIM-23244 (EC_{50} = 50 ± 33 pmol/L). BIM-23244 at a concentration of 10 nM induced a greater suppression of GH than octreotide at the same concentration (44 ± 5% *vs.* 26 ± 7%, respectively; $P < 0.014$). These results demonstrate that in the subclass of GH-secreting tumors responsive to both SSTR2- and SSTR5-preferential agonists (octreotide partial responders), the biselective BIM-23244 analog can achieve greater GH suppression than SSTR2-preferential drugs, such as octreotide.

Comparison between BIM-23244 and the combination of SSTR2- and SSTR5-preferential agonists (Fig. 4)

In the five cell cultures from adenomas equally sensitive to the SSTR2- and SSTR5- preferential agonists (octreotide partial responders), the dose-response inhibition of GH release by BIM-23244 was compared with that induced by a combination of the SSTR2 preferential agonist, BIM-23197, and the SSTR5 preferential agonist, BIM-23268, at equimolar doses. Similar maximal levels of GH suppression (44 ± 5%) were achieved by BIM-23244 and the combination of BIM-23197 and BIM-23268. The dose-response inhibitions of GH release induced by the two treatments were parallel. As expected from their respective IC_{50} values for both the SSTR2 and SSTR5 subtypes, the combination of BIM-23197 and BIM-23268 was slightly more potent in suppressing GH secretion than the biselective agonist BIM-23244.

Effect of BIM-23244 vs. octreotide on PRL release (Fig. 5)

In five tumor cell cultures (A1, A2, A7, A9, and A10), both PRL and GH were secreted into the culture medium. A dose-response inhibition of PRL secretion by SRIF-14 and by the different SRIF analogs was observed in all tumors, with a significant maximal inhibition of PRL release. As shown in

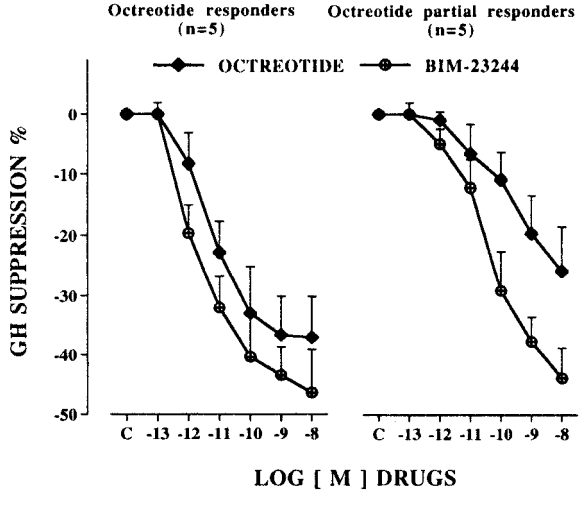

FIG. 3. Mean dose-response GH suppression curves obtained with octreotide and the SSTR2- and SSTR5-bispecific compound, BIM-23244 (10^{-13}–10^{-8} mol/L). Results are expressed as the mean ± SEM percent GH suppression *vs.* the control value (medium alone). The subclasses of octreotide responders (n = 5) or partial responders (n = 5) were defined by *in vivo* octreotide sensitivity, as shown in the Table 1, for each tumor.

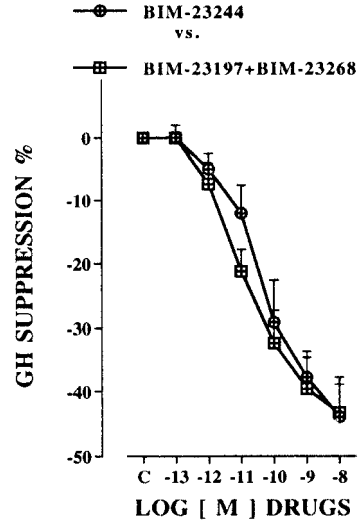

FIG. 4. Mean GH suppression dose-response curves with BIM-23244 alone and BIM-23197 in combination with BIM-23268 (10^{-13}–10^{-8} mol/L). Results are expressed as the mean ± SEM GH suppression *vs.* the control value (medium alone) in five SSTR2- and SSTR5-responsive adenomas.

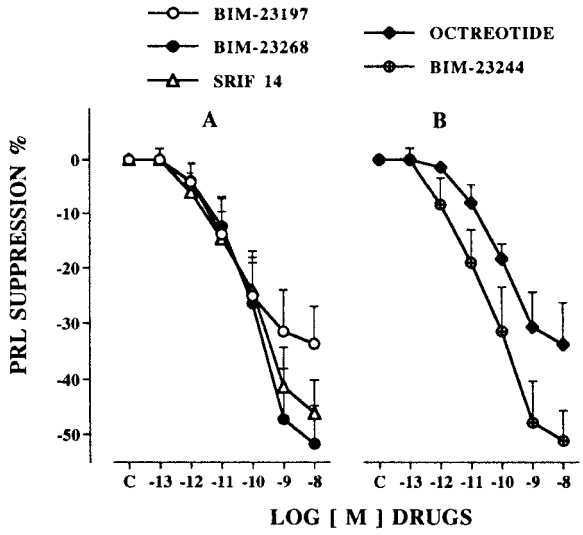

FIG. 5. Mean dose-response PRL suppression curves in five mixed GH- and PRL-secreting adenomas. A, With SRIF-14 and the SSTR2- and SSTR5-preferential compounds, BIM-23197 and BIM-23268, respectively. B, With octreotide and BIM-23244 (10^{-8}–10^{-13} mol/L). Results are expressed as the mean ± SEM percent PRL suppression *vs.* the control value (medium alone).

Fig. 5A, the dose-related inhibition of PRL release was similarly achieved with increasing concentrations of SRIF-14 and the SSTR5-preferring compound, BIM-23268. The SSTR2-preferring compound, BIM-23197, was partially effective in suppressing PRL suppression (mean maximal PRL inhibition, 34 ± 5% *vs.* 52 ± 6%, respectively, for BIM-23197 and BIM-23268). Compared with octreotide, the biselective analog, BIM-23244, was more effective in suppressing PRL secretion (Fig. 5B). The mean maximal PRL suppressions at 10 nmol/L BIM-23244 and octreotide were 51 ± 5% and 34 ± 7%, respectively ($P = 0.045$). These results in mixed GH-/PRL-secreting tumors indicate a better PRL-suppressive effect of either the SSTR5-preferring compound or the bispecific SSTR2 and SSTR5 compound compared with the agonists preferential for SSTR2 alone.

Discussion

The variable sensitivity of acromegalic patients to the current clinically available SRIF agonists, octreotide and lanreotide, has already been underlined. In previous studies of series of patients treated with increasing doses of octreotide (300–1500 μg/day) in 3 sc injections, levels of IGF-I normalized in 55% of the patients (12), whereas mean GH concentrations of 2 μg or less were only obtained in 22–26% of cases (3, 12). An improved patient response has been reported using the long-lasting depot formulations of either octreotide or lanreotide (13, 14). In these reports 70–80% of the acromegalic patients were considered to be controlled with these long-lasting SRIF agonists formulations. Such data were, in fact, biased due to preselection of patients already known to be responders through previous sc administration of octreotide. When such preselection is eliminated, the percentage of patients who achieve mean GH levels less than 2.5 μg/L with slow release lanreotide has been demonstrated in recent studies to be 50–60% (15). Thus, about 40–50% of acromegalic patients remain partially or poorly controlled under the current SRIF agonist treatments. In acromegaly, a quantitative loss of SRIF receptors explains the very poor or absent GH suppression in response to acute administration of octreotide or SRIF in 3 of 17 cases (4, 5). Such a loss of SRIF receptors is seldom encountered and cannot fully explain the partial GH-suppressive effects of octreotide and lanreotide *in vivo*. In a subsequent study of 37 GH-secreting tumors, the density of SRIF receptors was poorly correlated to the GH-suppressive effects of octreotide *in vivo* (16). Another hypothesis that could explain the partial GH-suppressive effects of octreotide or lanreotide in certain acromegalic patients comes from the identification of 5 SSTR subtypes (6). In human tumors of various origins, specific patterns of SSTR subtype expression have been described (17, 18). Among the GH-secreting adenomas, a consistent pattern of SSTR2 and SSTR5 mRNA expression has been identified (19–25). Previous studies have shown an inhibition of GH release using SSTR2-preferential agonists. However, the SSTR5-preferential agonist, BIM-23268, has also been shown to induce a significant inhibition of GH release in 7 of 15 GH-secreting tumors (8) and 6 of 7 GH-secreting tumors (7, 9). These data implicate the SSTR5 subtype in the inhibition of GH release in certain tumors. This hypothesis is confirmed in our study using the bispecific SSTR2- and SSTR5-preferential compound, BIM-23244. Indeed, when the tumors were only responsive to SSTR2 preferential analogs, this compound was unable to produce any additional effect on inhibition of GH release compared with octreotide. However, in the tumors equally responsive to both SSTR2 and SSTR5 agonists, BIM-23244 was significantly more potent than octreotide in the suppression of GH and PRL secretion. The comparison between dose-response inhibition of GH release with BIM-23244 and SRIF-14 showed that this compound more closely mimicked the effects of native SRIF by acting via both SSTR2 and SSTR5 subtypes.

From our data, two classes of tumors emerged among the GH-secreting adenomas. The first was a series of tumors characterized by high sensitivity to SRIF-14 and SSTR2-preferential agonists. These tumors presented the highest level of SSTR2 mRNA expression and had the highest GH-suppressive effect with octreotide. Why, despite equivalent SSTR5 mRNA expression, the SSTR5 preferential analog did not suppress GH release in such tumors remains unknown. In the second class of tumors, the level of SSTR2 mRNA was low, and octreotide produced only partial inhibition of GH release. SRIF-14 was nevertheless able to suppress GH release, with a maximal suppressive effect similar to that of the first class of tumors, but at a 10-fold higher concentration. The presence of high levels of SSTR5 mRNA was associated with a potent suppressive effect of BIM-23268 on GH release, more efficacious than that of the SSTR2 analogs. In these tumors, the bispecific SSTR2 and SSTR5 compound, BIM-23244, induced a suppression of GH release identical to that achieved by native SRIF. These data suggest that in tumors deficient in the SSTR2 subtype presenting with a high SSTR5/SSTR2 ratio there may be a rescue through the SSTR5 subtype that mediates the suppression of GH release.

Such changes in the intensity of GH suppression as a function of the specificity of the SRIF agonists are in keeping

with recent experimental data demonstrating ligand-induced SSTR subtype dimerization in CHO-K1 transfected cells (26). This study showed that the ligands (SRIF or SRIF agonist) could produce a homo- or heterodimerization of the SSTR receptor subtypes. Such a ligand-induced dimerization process resulted in increased binding affinity and modified SSTR functionality. Furthermore the ability of the ligands to homodimerize SSTR5 was highly dependent on the quantity of SSTR5 transfected into CHO-K1 cells. In our GH-secreting tumors presenting with high expression of SSTR2 mRNA, it could be that octreotide as well as ligands with high affinity to SSTR2 are inducing preferential SSTR2 homodimerization, mediating profound GH suppression in the picomolar range of these agonists. In the octreotide partially sensitive tumors, such a SSTR2 homodimerization could not be fully effective due to poor SSTR2 expression. In these cases, SSTR5 expressed at high levels could transduce GH inhibition by SRIF agonists with high affinity to SSTR5. Whether the SSTR5 preferential compounds act through homodimerization of SSTR5 subtypes or through SSTR5-SSTR2 receptor subtype heterodimerization remains speculative. Therefore, in octreotide partial responders in which the SSTR2 receptor is poorly efficient, the SSTR5-mediated pathway could compensate and transduce the inhibition of GH release in the presence of SSTR5 preferential compounds. The better efficacy of bipreferential SSTR2 and SSTR5, such as BIM-23244 and SRIF-14, compared with that of octreotide could support the hypothesis of a more efficient induced GH inhibition through SSTR5-SSTR2 heterodimerization.

In conclusion, the bispecific SRIF agonist, BIM-23244, targeting SSTR2 and SSTR5, was demonstrated to induce a greater GH- and PRL-suppressive effect in tumors considered partial octreotide responders. Such data, although significant in our cell culture studies, have to be extended by *in vivo* studies. Indeed, such an SSTR2- and SSTR5-bispecific agonist may also act upon other target cells bearing the SSTR5 subtype. Recent data (27) showed a preferential localization of SSTR1 and SSTR5 on pancreatic β-cells as well as a preferential inhibition of insulin release by the SSTR5 preferential compound, BIM-23268 (28, 29). It is thus mandatory, particularly in acromegalic patients, to assess *in vivo* the inhibition of insulin secretion that can be produced by administration of a SSTR2- and SSTR5-bispecific agonist.

Acknowledgments

We are grateful to Dr. P. M. Martin for using a Taq Man technology for real-time quantitative PCR in the Assistance Publique Hopitaux de Marseilles laboratory and to N. Peralez and Dr. D. Morando for technical assistance. The skillful help of Mrs. C. Taverna in the redaction of the manuscript was greatly appreciated.

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