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The Novel Somatostatin Analog SOM230 Is a Potent Inhibitor of Hormone Release by Growth Hormone- and Prolactin-Secreting Pituitary Adenomas *in Vitro*

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To determine the inhibitory profile of the novel somatostatin (SRIF) analog SOM230 with broad SRIF receptor binding, we compared the *in vitro* effects of SOM230, octreotide (OCT), and SRIF-14 on hormone release by cultures of different types of secreting pituitary adenomas. OCT (10 nM) significantly inhibited GH release in seven of nine GH-secreting pituitary adenoma cultures (range, −26 to −73%), SOM230 (10 nM) in eight of nine cultures (range, −22 to −68%), and SRIF-14 (10 nM) in six of six cultures (range, −30 to −75%). The sst analysis showed predominant but variable levels of somatostatin receptor $(sst)_2$ and sst_5 mRNA expression. In one culture completely resistant to OCT, SOM230 and SRIF-14 significantly inhibited GH release in a dose-dependent manner with an IC_{50} value in the low nanomolar range. In the other cultures, SOM230 showed a lower potency of GH release inhibition (IC_{50}, 0.5 nM), compared with OCT (IC_{50}, 0.02 nM) and SRIF-14 (IC_{50}, 0.02 nM). A positive correlation was found between sst_2 but not sst_5 mRNA levels in the adenoma cells and the inhibitory potency of OCT on GH release *in vivo* and *in vitro*, and the effects of SOM230 and SRIF-14 *in vitro*. In three prolactinoma cultures, 10 nM OCT weakly inhibited prolactin (PRL) release in only one (−28%), whereas 10 nM SOM230 significantly inhibited PRL release in three of three cultures (−23, −51, and −64.0%). The inhibition of PRL release by SOM230 was related to the expression level of sst_5 but not sst_2 mRNA. Several conclusions were reached. First, SOM230 has a broad profile of inhibition of tumoral pituitary hormone release in the low nanomolar range, probably mediated via both sst_2 and sst_5 receptors. The higher number of responders of GH-secreting pituitary adenoma cultures to SOM230, compared with OCT, suggest that SOM230 has the potency to increase the number of acromegalic patients which can be biochemically controlled. Second, compared with OCT, SOM230 is more potent in inhibiting PRL release by mixed GH/PRL-secreting adenoma and prolactinoma cells. (*J Clin Endocrinol Metab* 89: 1577–1585, 2004)

CURRENT TREATMENT OPTIONS in patients with acromegaly due to a GH-secreting pituitary adenoma are surgery, medical therapy, and radiotherapy. As medical therapy, stable somatostatin (SRIF) analogs, such as octreotide (OCT) and the sustained-release depot formulations Sandostatin-LAR and SR-lanreotide are widely used, both as primary or secondary therapy (1). Treatment with this generation of octapeptide SRIF analogs results in clinical and biochemical control, *i.e.* normalization of circulating GH and IGF-I levels, in approximately two thirds of the acromegalic patients (1). The successful medical treatment of acromegaly with octapeptide SRIF analogs is due to the expression of high-affinity (density) SRIF receptors on the adenoma cells, mainly somatostatin receptor $(sst)_2$, which is one of the five known SRIF receptor subtypes, $sst_{1–5}$, and the selective high-affinity binding of these analogs to the sst_2 subtype (2). The molecular basis for the clinical experience that one third of patients with acromegaly are not adequately controlled by treatment with octapeptide SRIF analogs is probably formed by a variable expression of $sst_{1–5}$ in the adenomas of these patients (3–9). Several groups have demonstrated that sst_2 and sst_5 receptors are the most important sst involved in the regulation of GH secretion (2, 9). Using sst selective SRIF analogs, it was shown that, in adenomas that were poorly responsive to the sst_2 selective agonists OCT and lanreotide, sst_5 selective or sst_2-sst_5 bispecific compounds were able to suppress GH release more potently (10, 11).

These observations initiated the development of novel stable SRIF analogs with a more universal sst binding profile. One of these new compounds, SOM230, was recently shown to reduce circulating IGF-I levels in rats by 75% after 126 d of continuous infusion. This effect was significantly more potent, compared with OCT, which suppressed IGF-I release under the same experimental condition by only 28% (12). In rats, the terminal elimination half-life was 23 h, which is approximately 11 times longer, compared with OCT (12). Finally, in both cynomolgus monkeys and beagle dogs, infusion of SOM230, and to a much lesser extent OCT (SMS 201–995), lowered IGF-I levels potently (13). Compared with OCT, SOM230 has a 30, 5, and 40 times higher binding affinity to sst_1, sst_3, and sst_5 receptors, respectively, and 2.5

Abbreviations: DA, Dopamine; hprt, hypoxanthine-phosphoribosyltransferase; OCT, octreotide; PRL, prolactin; SRIF, somatostatin; sst, somatostatin receptor.

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times lower affinity to sst_2 (Ref. 12 and Table 1). This universal binding profile of SOM230 to sst, in combination with the importance of sst_2 and sst_5 receptors in regulating GH secretion by pituitary adenoma cells, was the rationale for the present study to evaluate, for the first time, the effects of SOM230, OCT, and the native SRIF molecule SRIF-14 on hormone secretion by human GH-secreting pituitary adenomas. Moreover, recent evidence suggests that sst_5 receptors are also involved in the regulation of prolactin (PRL) secretion by human prolactinoma cells (11, 14). Therefore, we compared the effects of OCT and SOM230 on PRL secretion by primary cultures of human prolactinomas. A correlation is made with the sst subtype expression patterns in the adenoma cells.

Patients and Methods

Patients

Pituitary tumor samples were obtained by transsphenoidal operation from nine patients with GH-secreting pituitary adenomas and from three patients with prolactinomas as described in detail previously (15). Diagnosis was made on the basis of clinical and biochemical characteristics of the patients, in combination with (immuno)histochemistry of the tumor samples (Table 2). All patients gave their informed consent for the use of tumor material for research purposes. Directly after obtaining the tissue, a piece of tissue was snap frozen on dry ice and stored at −80 C until analysis. The remaining tissue was used for cell culture.

To evaluate *in vivo* responsiveness to OCT, patients received at 0900 h a single sc injection of 100 μg OCT. Blood samples were collected 30 min before and 1 min before, and hourly until 0800 h after sc injection. At the same time points, blood samples were taken at a control day. The effect of OCT on circulating GH levels was determined by calculating the mean GH suppression between 2 and 8 h after sc injection, compared with the same period of time on a control day.

Quantitative PCR

Quantitative PCR was performed as described previously (16). Briefly, poly A^+ mRNA was isolated using Dynabeads Oligo $(dT)_{25}$ (Dynal AS, Oslo, Norway) from freshly isolated pituitary adenoma cell pellets containing 0.5–1.0 × 10^6 adenoma cells. Analysis of sst subtype mRNA levels in both tissue or freshly isolated cell pellets (n = 4) yielded comparable results (data not shown). cDNA was synthesized using the poly A^+ mRNA, which was eluted from the beads in 40 μl H_2O for 10 min at 65 C, using Oligo $(dT)_{12–18}$ primer (Invitrogen, Breda, The Netherlands). One twentieth of the cDNA library was used for quantification of sst subtype mRNA levels. A quantitative PCR was performed by TaqMan Gold nuclease assay (Perkin-Elmer Corp., Foster City, CA) and the ABI PRISM 7700 sequence detection system (Perkin-Elmer) for real-time amplifications, according to manufacturer's protocol. The assay was performed using 15 μl TaqMan Universal PCR master mix (Applied Biosystems, Capelle aan de ijssel, The Netherlands), 500 nM forward primer, 500 nM reverse primer, 100 nM probe and 10 μl cDNA template, in a total reaction volume of 25 μl. The reactions were carried out in a ABI 7700 sequence detector (Perkin-Elmer, Groningen, The Netherlands). After an initial heating at 95 C for 8 min, samples were subjected to 40 cycles of denaturation at 95 C for 15 sec and annealing for 1 min at 60 C. The primer and probe sequences that were used are indicated below. The detection of hypoxanthine-phosphoribosyl-transferase (hprt) mRNA served as a control and was used for normalization of the sst subtype mRNA levels.

The primer sequences that were used included the following: hprt forward, 5′-TGCTTTCCTTGGTCAGGCAGTAT-3′; hprt reverse, 5′-TCAAATCCAACAAAGTCTGGCTTATATC-3′; sst_1 forward, 5′-TGAGTCAGCTGTCGGTCATC-3′; sst_1 reverse, 5′-ACACTGTAGGCACGGCTCTT-3′; sst_2 forward, 5′-TCGGCCAAGTGGAGGAGAC-3′; sst_2 reverse, 5′-AGAGACTCCCCACACAGCCA-3′; sst_3 forward, 5′-CTGGGTAACTCGCTGGTCATCTA-3′; sst_3 reverse, 5′-AGCGCCAGGTTGAGGATGTA-3′; sst_5 forward, 5′-CATCCTCTCCTACGCCAACAG-3′; and sst_5 reverse, 5′-GGAAGCTCTGGCGGAAGTT-3′.

The probe sequences that were used included the following: hprt, 5′-FAM-CAAGCTTGCGACCTTGACCATCTTTGGA-TAMRA-3′; sst_1, 5′-FAM-ACAGCTGCGCCAACCCCATC-TAMRA-3′; sst_2, 5′-FAM-CCGGACGGCCAAGATGATCACC-TAMRA-3′; sst_3, 5′-FAM-CGGCCAGCCCTTCAGTCACCAAC-TAMRA-3′; and sst_5, 5′-FAM-CCCGTCCTCTACGGCTTCCTCTCTGA-TAMRA-3′.

Primers and probes were purchased from Biosource (Nivelles, Belgium).

The relative amount of sst subtype mRNA was determined using a

TABLE 1. Binding affinity of SRIF-14, OCT, and SOM230 for the five human sst, $sst_{1–5}$

Compound	$hsst_1$	$hsst_2$	$hsst_3$	$hsst_4$	$hsst_5$
SRIF-14	0.93 ± 0.12	0.15 ± 0.02	0.56 ± 0.17	1.5 ± 0.4	0.29 ± 0.04
OCT	280 ± 80	0.38 ± 0.08	7.1 ± 1.4	>1000	6.3 ± 1.0
SOM230	9.3 ± 0.1	1.0 ± 0.1	1.5 ± 0.3	>100	0.16 ± 0.01

Data are reproduced with permission (12). Results are the mean ± SE; IC_{50} values are expressed in nanomolar concentration.

TABLE 2. Patient and hormone data of the 12 patients with pituitary adenoma

Patient no.	Sex (M/F)	Age (yr)	GH (μg/liter)	PRL (μg/liter)	IGF-I (nmol/liter)	*In vitro* hormone release	
						GH (ng/10^5 cells·72 h)	PRL (ng/10^5 cells·72 h)
GH-secreting adenoma							
1	M	58	9.8	19	175	520 ± 24	1192 ± 60
2	M	26	31	111	201	547 ± 7	492 ± 7
3	F	60	5.4	39	66	372 ± 13	1105 ± 71
4	F	55	15	19	184	1021 ± 19	–
5	M	36	118	24	111	336 ± 35	–
6	F	44	14	15	289	230 ± 10	–
7	F	41	72	6.7	285	640 ± 18	–
8	F	42	6.8	0.9	88.3	55 ± 2	21 ± 2
9	F	65	6.8	9.3	169	246 ± 9	156 ± 5
Prolactinoma							
10	F	35		2000		–	5638 ± 143
11	F	64		13520		–	1486 ± 80
12	F	37		32		–	1700 ± 50

Normal range for IGF-I, 12–40 nmol/liter. M, Male; F, female; –, not detectable.

standard curve generated from known amounts of human genomic DNA. For determination of the amount of hprt mRNA, a standard curve was generated of a pool of cDNAs from a human cell line known to express hprt. The linear range of amplification ranged between 4 log dilutions of genomic DNA or cDNA, respectively. The relative amount of sst subtype mRNA was calculated relative to the amount of hprt mRNA and is given in arbitrary units. Each sample was assayed in duplicate.

Cell dispersion and cell culture

Single-cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as described in detail previously (15). For short-term incubation of monolayer cultures, the dissociated cells were plated in 48-well plates (Corning, Cambridge, MA) at a density of 10^5 cells per well per 1-ml culture medium. After 3–4 d the medium was changed and 72-h incubations without or with test substances were initiated. At the end of the incubation, the medium was removed and centrifuged for 5 min at 600 × *g*. The supernatant was collected and stored at −20 C until analysis. The choice for a 72-h incubation was made on the basis of previous studies, in which we demonstrated that exposure of GH-secreting pituitary adenoma cells for 4–96 h to octreotide showed a variable, but in all instances during longer incubations statistically significant inhibition of GH release, which paralleled the sensitivity of GH secretion to octreotide *in vivo* (17). For long-term incubation studies in Transwells (18), the isolated tumor cells were plated in Transwell-COL membranes (Corning) at a density of 10^5 cells/well. The Transwells were then placed into multiwell plates (24-well, Corning) containing 1 ml culture medium. After 72 h the Transwells were transferred to wells containing fresh medium (without or with test substances). Every 3–4 d the cells were placed into fresh medium and the incubation media were collected and stored at −20 C until determination of hormone concentrations.

The cells were cultured at 37 C in a CO_2 incubator. The culture medium consisted of MEM supplemented with nonessential amino acids, sodium pyruvate (1 mmol/liter), 10% fetal calf serum, penicillin (1×10^5 U/liter), fungizone (0.5 mg/liter), L-glutamine (2 mmol/liter), and sodium bicarbonate (2.2 g/liter, pH 7.6). Media and supplements were obtained from Gibco Bio-cult Europe (Invitrogen, Breda, The Netherlands).

Unfortunately, generally not enough tumor material was obtained to test for each tumor the dose dependency of effects for the indicated drugs.

Hormone determinations

Human GH and PRL concentrations in the media and cell extracts were determined by a nonisotopic, automatic chemiluminescence immunoassay system (Immulite; Diagnostic Products Corp. Inc., Los Angeles, CA). Intra- and interassay coefficients of variation for GH and PRL were 6.0 and 5.7% and 6.2 and 6.4%, respectively. ACTH, LH, and FSH concentrations in the culture media were determined as well to exclude the presence of contaminating normal pituitary cells in the cultures. Human ACTH, LH, and FSH concentrations were determined by a nonisotopic, automatic chemiluminescence immunoassay system (Immulite, Diagnostic Products Corp. Inc.). Intra- and interassay coefficients of variation for ACTH, LH, and FSH were 5.6, 5.7, and 6.4% and 7.8, 12.3, and 7.5%, respectively. Except for the expected hormones GH and PRL, none of the other hormones were detectable (not shown).

Test substances

OCT (Sandostatin) and bromocriptine were obtained from Novartis Pharma A.G. (Basel, Switzerland). SOM230 was provided by Novartis Pharma A.G. Somatostatin-14 (SRIF-14) was purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis of the data

All data on hormone release are expressed in mean ± SE, n = 4 wells per treatment group. All data were analyzed by ANOVA to determine overall differences between treatment groups. When significant differences were found by ANOVA, a multiple comparison between treatment groups was made using the Newman-Keuls test. Correlation analysis was done by the use of the Spearman's rank correlation test. Calculation of IC_{50} values for inhibition of hormone release were made using GraphPad Prism (San Diego, CA).

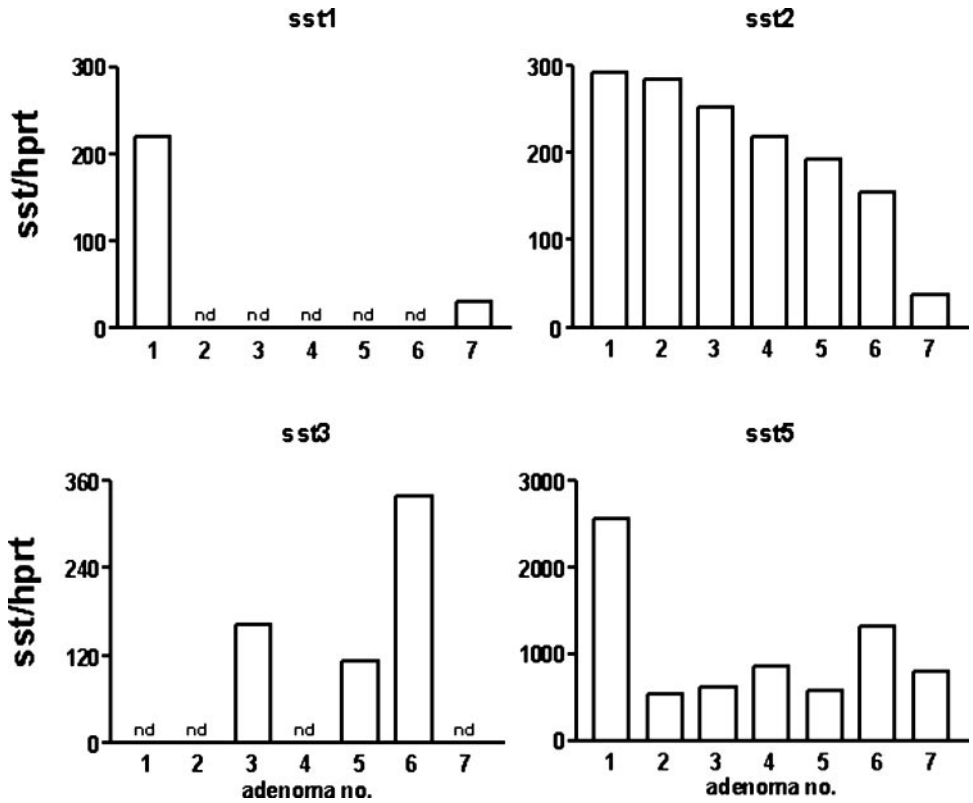

FIG. 1. Relative sst_1, sst_2, sst_3, and sst_5 mRNA expression levels in GH-secreting pituitary adenomas. Values are expressed as the number of copies of the respective sst mRNAs, relative to the number of copies of hprt. nd, Not detectable. Note the differences in the scale of the y-axis between sst_1, sst_2, and sst_3 mRNA levels on the one hand, and the higher sst_5 mRNA levels on the other hand. The relative amount of sst subtype mRNA was calculated relative to the amount of hprt mRNA and is given in arbitrary units.

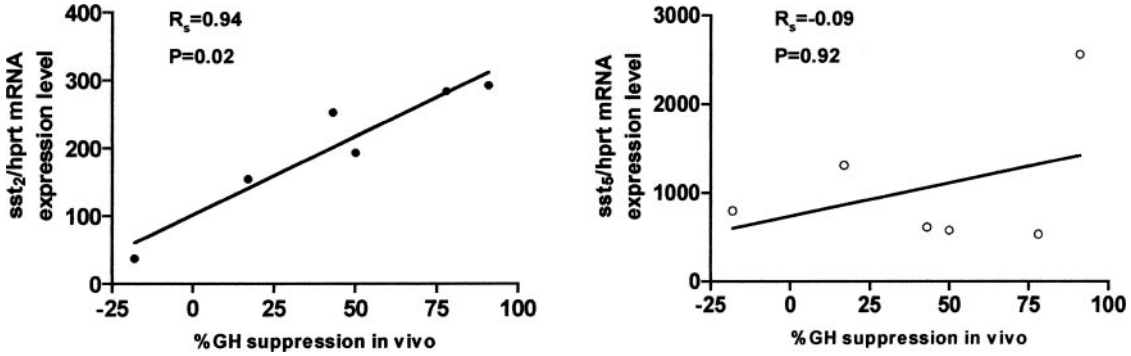

FIG. 2. Correlation between sst_2 and sst_5 mRNA levels and the *in vivo* response of GH release to a single sc injection of 100 μg OCT in six acromegalic patients. The relative amount of sst subtype mRNA was calculated relative to the amount of hprt mRNA and is given in arbitrary units.

Results

Sst subtype mRNA expression and correlation with in vivo GH suppression by octreotide

In seven of nine GH-secreting pituitary adenoma samples the sst subtype mRNA expression pattern was determined. Figure 1 shows that two adenomas expressed sst_1 mRNA, three adenomas expressed sst_3 mRNA and that all adenomas expressed sst_2 and sst_5 mRNAs. Expression of sst mRNAs was variable between adenoma samples. The difference between the lowest and the highest level measured, amounted to 7-, 8-, 3-, and 5-fold for sst_1, sst_2, sst_3, and sst_5, respectively. The sst subtype mRNA levels showed no statistical correlation (data not shown). The mean percentage *in vivo* GH suppression (range +18 and −91% suppression) between 2 and 8 h after the administration of a single sc dose of 100 μg OCT (patients 1, 2, 3, 5, 6, and 7) was positively correlated with sst_2 mRNA expression levels (Fig. 2, *left panel*) but not with sst_5 mRNA levels (Fig. 2, *right panel*).

Effects of SS analogs on GH secretion

In most GH-secreting pituitary adenomas, the effect of 10 nM OCT, SOM230, or SRIF-14 on GH release was evaluated in parallel *in vitro*. Figure 3 shows that GH release was significantly suppressed by OCT in seven of nine cultures, by SOM230 in eight of nine cultures and by SRIF-14 in six of six cultures. In the majority of the adenomas, the percentage suppression using a maximally active concentration of the three compounds, was comparable. Only the adenoma cells of patient 7 showed a dissociated response to OCT on the one hand and to SOM230 and SRIF-14 on the other hand. In this adenoma culture, GH release was not inhibited by OCT, whereas both SOM230 and SRIF-14 induced a statistically significant suppression of GH release. In the respective patient, OCT (100 μg sc) did not suppress GH concentrations as well. As seen in Fig. 3, the response in terms of GH suppression was variable between the individual adenoma cultures. This variable responsiveness to OCT and SOM230 correlated well with the sst_2 mRNA expression levels in the adenoma cells of the respective patients (Fig. 4, *upper panel*). Comparable to the patients responsiveness to OCT, sst_5 mRNA expression showed no statistically significant correlation with the percentage of GH suppression by 10 nM OCT.

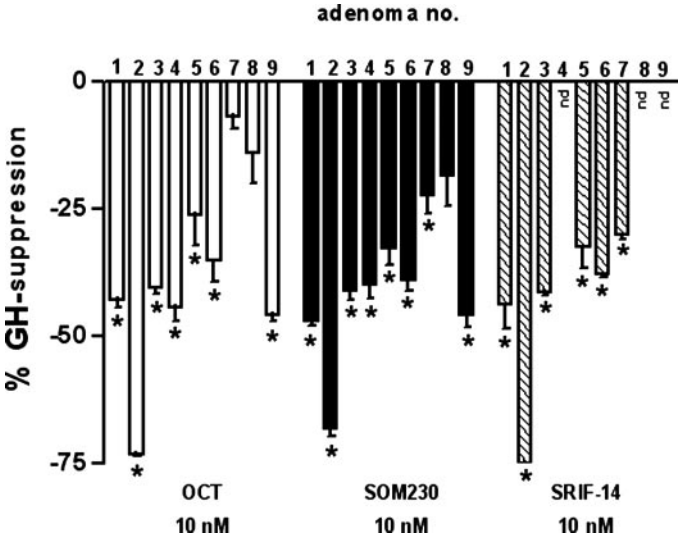

FIG. 3. Effects of OCT (10 nM; *open bars*), SOM230 (10 nM; *filled bars*), and SRIF-14 (10 nM; *hatched bars*) on GH release by nine cultured GH-secreting pituitary adenomas. The results are expressed as the percentage inhibition of GH release, compared with untreated, control cells. *, $P < 0.01$ *vs.* untreated control cells. nd, Not done.

Interestingly, the effects of 10 nM SOM230, which shows a more universal binding profile for sst receptors, including high-affinity binding to sst_5, also showed no correlation with sst_5 mRNA expression levels. This is demonstrated in Fig. 4 (*lower panel*).

In several adenoma cultures, the dose-response relationship of the inhibitory effects of OCT, SOM230, and SRIF-14 could be evaluated. Figure 5 (*right panel*) shows the effects of the three compounds on GH release by the adenoma cells of patient 7, which did not respond to OCT. The adenoma cells of this patient had the lowest sst_2 mRNA levels of all cases studied (Fig. 1, *upper right panel*). As shown, GH release by the cells of this patient was inhibited in a dose-dependent manner by both SOM230 and SRIF-14. The IC_{50} values for the inhibition of GH release were 0.5 and 0.6 nM for SOM230 and SRIF-14, respectively. In the other adenoma cultures, both OCT and SRIF-14 were slightly more efficacious, in terms of IC_{50} values, compared with SOM230. Figure 5 (*left panel*) shows the mean dose response of OCT, SOM230, and SRIF-14 for the other cultures. Mean IC_{50} values were 0.02, 0.5, and 0.02 nM for OCT, SOM230, and SRIF-14, respectively.

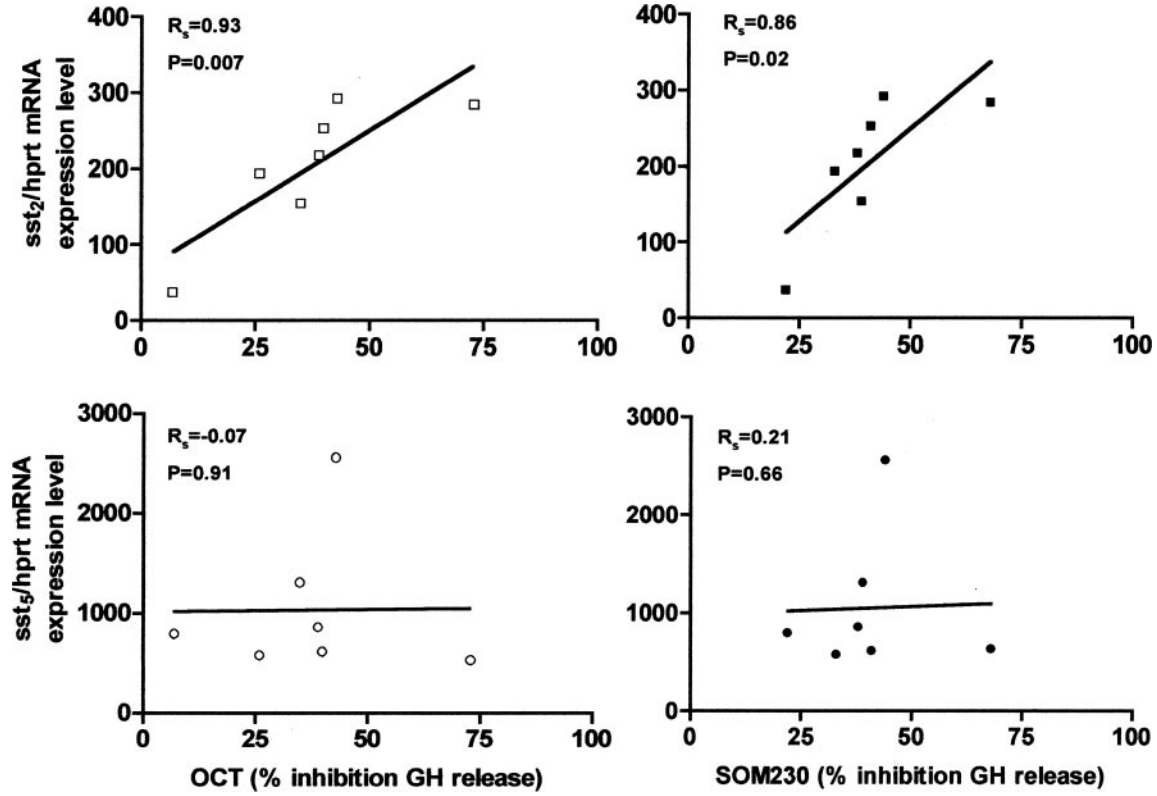

FIG. 4. Correlation between sst_2 (*upper panel*) and sst_5 (*lower panel*) mRNA levels and the percentage suppression of GH release induced by SOM230 (10 nM) and OCT (10 nM). The relative amount of sst subtype mRNA was calculated relative to the amount of hprt mRNA and is given in arbitrary units.

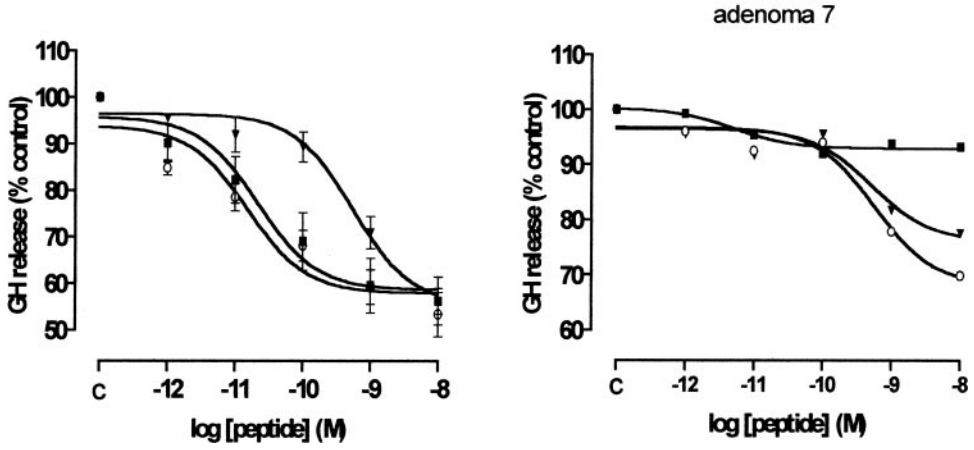

FIG. 5. Dose-dependent inhibition of GH release by SOM230 (▼), OCT (■), and SRIF-14 (○) in cultured pituitary adenoma cells. The *left panel* represents the mean dose-dependent effects of SOM230 (n = 5; adenoma no. 2, 3, 4, 5, and 6), OCT (n = 4; adenoma no. 2, 3, 5, and 6), and SRIF-14 (n = 4; adenoma no. 2, 3, 5, and 6). The *right panel* shows the dose-dependent inhibitory effects of the compounds on GH release by cells patient 7.

Effects of somatostatin (SS) analogs on PRL secretion

Because previous studies demonstrated the involvement of sst_5 receptors in regulating PRL release, we also compared the effects of OCT and SOM230 on PRL release. Five adenoma cultures cosecreted PRL as well. OCT (10 nM) and SOM230 (10 nM) significantly inhibited PRL release in four of five and five of five cultures, respectively (Fig. 6). The percentage suppression was between 16 and 66% for OCT and between 38 and 74% for SOM230, indicating its higher efficacy. In four of five cultures (no. 1, 3, 8, and 9), SOM230 was significantly more potent with regard to its maximal suppressive effect on PRL release, when compared with OCT ($P < 0.01$).

In addition to mixed GH/PRL-secreting pituitary adenomas, we also compared the effects of OCT and SOM230 on PRL release in three primary human prolactinoma cultures (no. 10, 11, and 12) (Table 2). Figure 7 shows that prolactinoma no.10 selectively expresses a high level of sst_5 mRNA, whereas prolactinoma 11 expressed sst_1 mRNA but very low sst_5 mRNA. In agreement with the low affinity of OCT for sst_5 and the very high affinity of SOM230 for this sst, 10 nM OCT did not significantly inhibit PRL release by the two prolactinoma cultures, whereas 10 nM SOM230 potently suppressed (−49%) PRL release by cells of adenoma 10 (high sst_5) and slightly (−23%) by cells of prolactinoma no.11 (low sst_5). Both adenoma cultures showed high sensitivity to 10 nM of

the dopamine agonist bromocriptine (−73 and −89% inhibition in cultures 10 and 11, respectively). In one prolactinoma culture (no. 12) the long-term *in vitro* effects of OCT, SOM230, and bromocriptine were studied. Figure 8 shows that both SOM230 (10 nM) and bromocriptine (10 nM) suppressed PRL release by approximately 90% after 9 d of continuous incubation with the compounds. In this particular adenoma, OCT was only slightly effective (−30% after 9 d). Unfortunately, not enough tissue was obtained from this patient to study the sst subtype expression pattern.

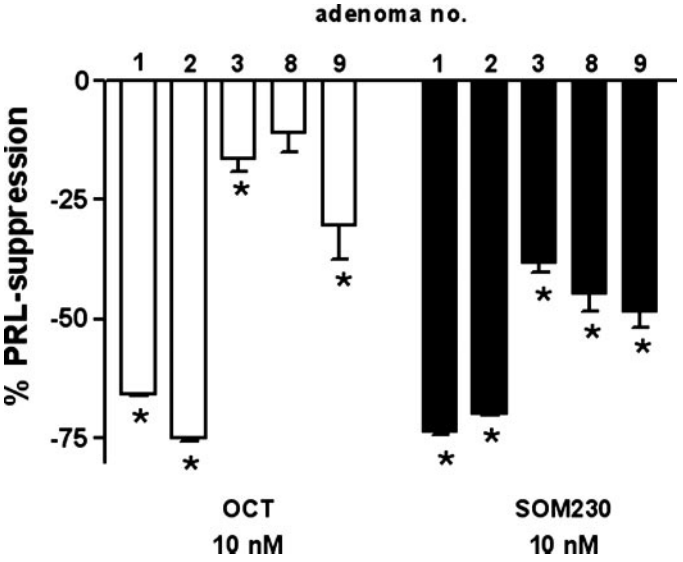

FIG. 6. Effect of 10 nM OCT (*open bars*) or 10 nM SOM230 (*filled bars*) on PRL secretion by mixed GH/PRL-secreting pituitary adenomas. The results are expressed as the percentage inhibition of PRL release, compared with untreated, control cells. *, $P < 0.01$ *vs.* untreated control cells.

Discussion

SRIF receptor expression on GH-secreting pituitary adenomas forms the basis for successful treatment of acromegalic patients using sst_2-selective octapeptide SRIF-analogs (19, 20). Recent *in vitro* studies have demonstrated that both sst_2 and sst_5 receptors are involved in the regulation of GH release by normal human fetal anterior pituitary cells and GH-secreting pituitary adenoma cells (2, 9, 11). In addition, evidence exists that sst_5 exclusively regulates PRL secretion from human prolactinoma cells (11, 14). Moreover, BIM-23244, a SRIF analog with high-affinity binding to sst_2 and sst_5 receptors, was shown to achieve a better suppression of GH secretion by cultured GH-secreting pituitary adenomas that were partially responsive to OCT, suggesting that such bispecific SS analogs could achieve a better control of GH secretion in a larger number of acromegalic patients (10). The recent observation that sst subtypes may form homo- and heterodimers, resulting in receptors with enhanced binding affinity and modified functional properties (21, 22), may form one of the explanations for the enhanced efficacy of bispecific compounds such as BIM-23244. In addition, an sst_2 antagonist was shown to inhibit the GH suppressing action of sst_2/sst_5 biselective agonists in human fetal pituitary cells, supporting the concept of functional interaction between sst_2 and sst_5 receptors in the regulation of GH secretion (23).

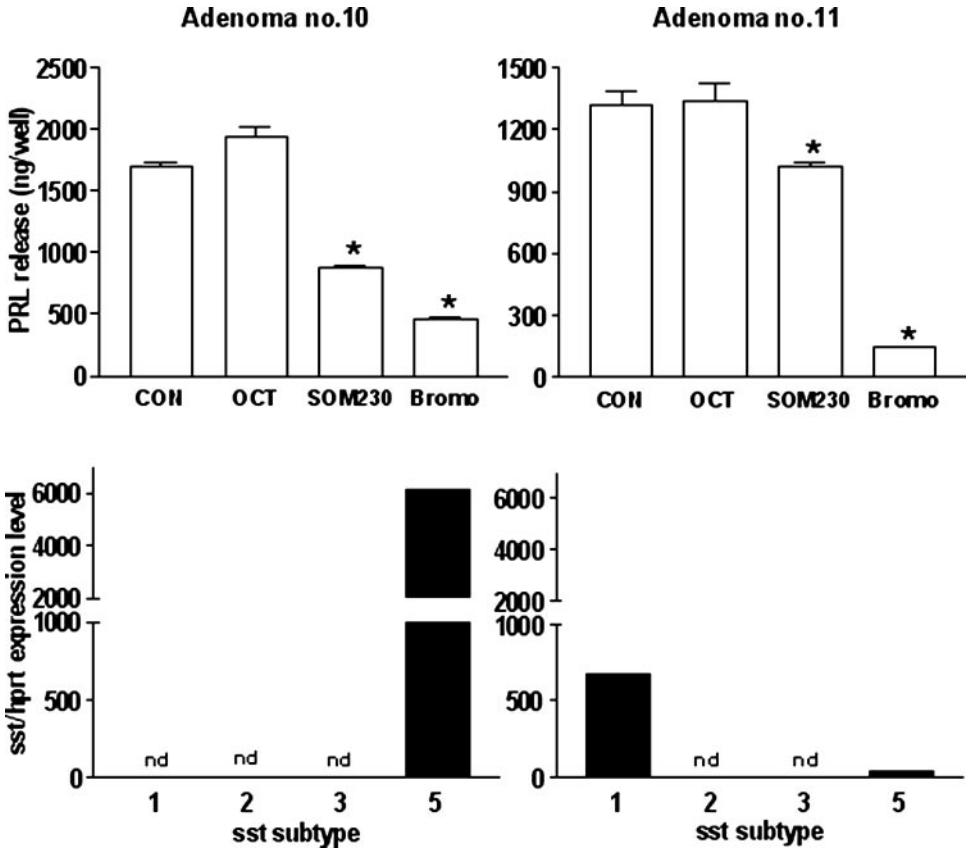

FIG. 7. *Upper panel*, Effects of SOM230 (10 nM), OCT (10 nM), and bromocriptine (10 nM) on PRL release by two cultured prolactinomas (no. 10 and 11). *, $P < 0.01$ *vs.* untreated control cells (incubation time 72 h). *Lower panel*, sst subtype mRNA expression in the adenoma cells of patient 10 and 11.

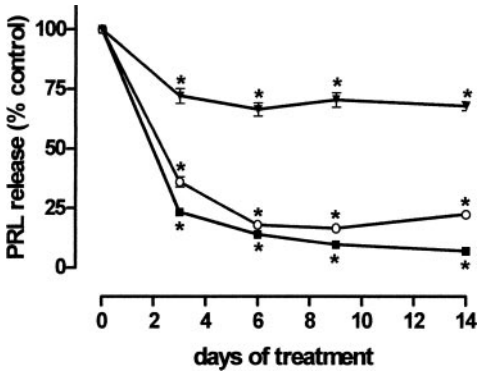

FIG. 8. The effect of long-term *in vitro* incubation with OCT (10 nM), SOM230 (10 nM), and bromocriptine (10 nM) on PRL release by cultured prolactinoma cells of patient no. 12. The pituitary adenoma cells were cultured in Transwell tissue culture inserts for 14 d without or with the compounds indicated. Medium was collected and refreshed every 3 or 4 d and stored at −20 C until determination of PRL concentrations. Values are expressed as the percentage of hormone release of control cells at each time point indicated and are the mean ± SEM of four wells per treatment group. ▼, OCT; ○, SOM230; ■, bromocriptine. *, $P < 0.01$ *vs.* untreated control cells (incubation time 72 h).

Recently a novel stable SRIF analog, with a more universal binding profile and high-affinity binding to sst_1, sst_2, sst_3, and sst_5, was introduced (12, 13). This SRIF analog, SOM230, was shown to be significantly more potent in lowering IGF-I levels in rats, compared with the sst_2-preferring analog OCT (12). SOM230 has a favorable elimination half-life of 23 h (12), suggesting that this analog may be suitable for clinical application as well (24). On the basis of the involvement of sst_2/sst_5 receptors in regulating GH secretion and sst_5 receptors in regulating PRL secretion, we compared in the present study the efficacy of SOM230 in the regulation of GH and PRL release by primary cultures of human pituitary adenoma cells.

In agreement with previous studies (3–10), we found a variable expression of sst mRNAs, predominantly sst_2 and sst_5, in our series of GH-secreting pituitary adenomas. Only selected cases expressed sst_1 and sst_3 mRNAs, suggesting that these sst subtypes are probably of less importance in this type of pituitary adenoma. Moreover, in most adenomas, sst_5 expression levels were relatively higher, compared with sst_2 mRNA levels. On the other hand, the preoperative response of acromegalic patients to a single sc administration of 100 μg OCT was positively correlated with the sst_2 mRNA, but not with sst_5 mRNA levels, in the pituitary adenomas of the patients obtained via transsphenoidal surgery. These data demonstrate that the sst_2 subtype is clearly the predominant receptor determining *in vivo* responsiveness to OCT in acromegalic patients.

In agreement with previous observations (10), sst_2 mRNA levels were positively correlated with the *in vitro* GH suppression by this sst_2 selective SRIF analog as well. Surprisingly, although SOM230 shows a very high affinity for sst_5 receptors, no significant correlation was found between the *in vitro* GH suppression by a maximally active concentration of SOM230 and sst_5 mRNA levels, whereas a positive correlation was found between the effects of SOM230 and sst_2 mRNA levels. Although these data were unexpected, a previous study by Jaquet *et al.* (9) in fact already made the same observation using SRIF-14. In this study, sst_2 mRNA but not sst_5 mRNA levels in GH-secreting pituitary adenomas showed a positive correlation with *in vitro* GH suppression by 1 nmol/liter SRIF-14.

As suggested by Jaquet *et al.* (9), it is not known yet whether the observed sst mRNA levels directly correlate with sst protein levels. The importance of the sst_2 subtype in regulating GH secretion by the majority of human GH-secreting pituitary adenomas is also evident by our observation that the potency of OCT in terms of IC_{50} values measured for the inhibition of GH release are slightly higher, compared with SOM230. Nevertheless, SOM230 shows a high efficacy to inhibit GH release, with an IC_{50} value in the low nanomolar range. In 89% of the cultures SOM230 significantly inhibited GH release. In this respect, OCT is slightly less efficacious, with a significant GH-suppressive effect in seven of nine cultures (78%). In one OCT-resistant culture, SOM230 inhibited GH release with an IC_{50} value comparable with that of SRIF-14 (IC_{50}, 0.5 and 0.6 nM, respectively), confirming the importance of the sst_5 receptor subtype in mediating GH release, when sst_2 levels are low. However, although SOM230 has a slightly lower potency (IC_{50}) for the inhibition of GH release in most cases, compared with OCT, its efficacy is higher, compared with OCT, in terms of the number of responders. Therefore, in addition to the sst_2/sst_5 bispecific compound BIM-23244, SOM230 also has the potential to achieve better control of GH hypersecretion in a larger number of acromegalic patients. Moreover, the very favorable elimination half-life of SOM230 (12) makes this compound an interesting candidate for clinical application as well.

Apart from regulating GH secretion, sst_5 receptors play a regulatory role in normal and tumoral PRL secretion as well (2, 11, 14). A significant proportion of GH-secreting pituitary adenomas contain GH- and PRL-expressing cells, either as individual cells expressing GH or PRL or as mammosomatotroph cells expressing GH and PRL in the same cells (25). In agreement with these data, we observed cosecretion of GH and PRL in 56% of the cultures and showed that SOM230 has a potent inhibitory effect on PRL secretion in mixed GH-PRL-secreting pituitary adenomas and prolactinomas. In four of five mixed GH-PRL-secreting pituitary adenomas, SOM230 was significantly more potent, compared with OCT, in its maximal suppressive effect on PRL secretion. Recently it was shown that activation of sst_1 by the sst_1-selective agonist BIM-23296 caused a dose-dependent inhibitory effect in the nanomolar range on GH and PRL secretion by GH-secreting pituitary adenomas. In addition to lowering GH and PRL secretion, this SS-analog induced a decrease in cell viability as well (26). In our series we found that two of seven adenomas expressed sst_1. In selected cases, therefore, sst_1 may play a regulatory role on GH-secreting pituitary adenoma cell function as well.

In three dopamine (DA) agonist-sensitive prolactinomas, SOM230 was significantly more potent than OCT in lowering PRL secretion. In two of the prolactinomas, there was a clear relationship between the expression of sst_5 mRNA in the adenoma cells and the percentage inhibition of PRL secretion by SOM230. In one prolactinoma culture, which expressed

high levels of sst_5 mRNA and no other sst mRNAs, PRL secretion was reduced to the same extent as that induced by bromocriptine. One other prolactinoma, which showed a significantly lower responsiveness to SOM230, had very low sst_5 mRNA levels. The lower potency of OCT in reducing PRL secretion by prolactinomas seems related to the very low sst_2 levels, as was demonstrated in a series of 10 prolactinomas by Jaquet *et al.* (14). These data further underline the role of sst_5 in mediating its suppressive effect on PRL secretion. However, the potential clinical importance of these findings should be considered in view of the very high proportion of patients with prolactinomas responding to DA agonist treatment with a normalization of PRL levels and tumor shrinkage (27, 28). In addition, Jaquet *et al.* (14) previously showed that the effects of sst_5 selective compounds on prolactinoma cells are superimposable at higher concentration to those of the DA agonists, but not additive, particularly in adenomas resistant to dopaminergic suppression of PRL release.

Whereas sst_5 receptors may mediate antiproliferative effects (29), the role of this receptor in the control of pituitary adenoma cell proliferation is unclear. Indirect evidence for a role of sst_5 in the regulation of human GH-secreting pituitary adenoma cell proliferation was obtained from the observation of a germ line mutation (Arg240Trp) in genomic DNA from pituitary adenoma and peripheral blood mononuclear cells of an acromegalic patient resistant to SRIF analog treatment and a higher proliferation rate of cells overexpressing this mutant sst_5 receptor, compared with cells expressing wild-type sst_5 (30). Whether activation of sst_5 receptors expressed on human GH-secreting pituitary adenomas and prolactinomas also mediates an antiproliferative effect in these cell types remains to be elucidated, however.

Because somatostatin analogs inhibit the secretion of insulin, impaired glucose tolerance was observed after the acute administration of octreotide (31). Recently we observed similar glucose responses to the acute administration of SOM230 *in vivo* (32). However, SOM230 did not modify insulin secretion, suggesting another mechanism of action. The inhibitory effect of octreotide on insulin secretion is short-lived, and clinically important effects on carbohydrate metabolism during long-term therapy are not observed (33). Although the acute rise in glucose levels after SOM230 injection requires further attention, preclinical studies in cynomolgus monkeys showed that insulin, glucagon, and glucose levels remained unchanged after 7 d of high-dose infusion with SOM230 (13). In addition, 126 d treatment of rats with pharmacological doses of SOM230 did not modify plasma glucose levels (12). These data suggest that SOM230 is well tolerated in rats and monkeys with regard to glucose homeostasis.

In conclusion, the novel universal SRIF analog SOM230 is a potent inhibitor of GH and PRL secretion in GH-secreting pituitary adenomas. The higher number of cultures responding to SOM230, compared with OCT, suggests that SOM230 has the potential to increase the number of patients controlled biochemically, both via sst_2 and sst_5. In addition, SOM230 is more potent in its inhibitory effect on PRL secretion in mixed GH/PRL-secreting pituitary adenomas. In prolactinoma cultures, sst_5 receptors mediate the potent inhibitory effects of SOM230 on PRL secretion. Because the majority of patients with prolactinomas are successfully treated using DA agonists, there may be a role for SOM230 in the treatment of prolactinoma patients, which are intolerant to DA agonists.

Acknowledgments

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