0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(2):638–645

doi: 10.1210/jc.2003-031052

A Single-Dose Comparison of the Acute Effects between the New Somatostatin Analog SOM230 and Octreotide in Acromegalic Patients

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Treatment with the somatostatin receptor (sst) subtype 2 predominant analogs octreotide and lanreotide induces clinical and biochemical cure in approximately 65% of acromegalic patients. GH-secreting pituitary adenomas, which are not controlled, also express sst_5. We compared the acute effects of octreotide and SOM230, a new somatostatin analog with high affinity for $sst_{1,2,3,5}$ on hormone release in acromegalic patients. In a single-dose, proof-of-concept study, 100 μg octreotide and 100 and 250 μg SOM230 were given sc to 12 patients with active acromegaly. Doses of 100 and 250 μg SOM230 dose-dependently suppressed GH levels from 2–8 h after administration (−38 ± 7.7 *vs.* −61 ± 6.7%, respectively; $P < 0.01$). A comparable suppression of GH levels by octreotide and 250 μg SOM230 was observed in eight patients (−65 ± 7 *vs.* −72 ± 7%, respectively). In three patients, the acute GH-lowering effect of 250 μg SOM230 was significantly superior to that of octreotide (−70 ± 2 *vs.* −17 ± 15%, respectively; $P < 0.01$). In one patient, the GH-lowering effect of octreotide was better than that of SOM230. Tolerability for SOM230 was good. Glucose levels were initially slightly elevated after octreotide and SOM230, compared with control day, whereas insulin levels were only significantly suppressed by octreotide. We conclude that SOM230 is an effective GH-lowering drug in acromegalic patients with the potential to increase the number of patients controlled during long-term medical treatment. (*J Clin Endocrinol Metab* 89: 638–645, 2004)

IN THE MAJORITY of patients, acromegaly is caused by a GH-secreting pituitary adenoma, resulting in high circulating GH and IGF-I concentrations. The first choice of medical treatment are the somatostatin (SS) analogs, a safe and effective strategy, mimicking the action of the native peptide SS in its inhibitory effect on GH release by the adenoma cells (1). The first clinically available SS analog octreotide (OCT) has been shown to be effective as primary or secondary therapy for acromegalic patients (2–5). Several studies have demonstrated that long-term therapy with OCT or lanreotide, administered either sc or as a long-acting depot preparation by im injection, induced clinical and biochemical cure in approximately 65% of patients (6–10). Still, a significant percentage of GH-secreting pituitary tumors seems relatively resistant to OCT and lanreotide, and this may be explained in part by a variable tumoral expression or reduced receptor density of the five known SS receptor (sst) subtypes on the adenomas of these patients (11). Functional evidence for the existence of sst subtypes comes from studies using human fetal pituitary cell cultures in which SS regulates GH and TSH secretion mainly by sst_2 and sst_5 and prolactin (PRL) secretion mainly by sst_5 (12). Most GH-secreting pituitary adenomas predominantly express mRNA for sst_2 and sst_5, whereas sst_1 and sst_3 are moderately expressed and sst_4 not found (13, 14). SS binds with high affinity to all five sst subtypes, whereas OCT and lanreotide display a high, low, and moderate affinity to sst_2, sst_{1+4}, and sst_{3+5}, respectively. Saveanu *et al.* (15) compared the *in vivo* sensitivity of GH release for OCT in nine acromegalic patients with the tumor mRNA expression for sst_2 and sst_5 subtypes. It was observed that sst_2 mRNA expression was lower, and sst_5 mRNA was higher in adenomas that were partially sensitive to OCT, compared with OCT-sensitive adenomas. In the group of partially OCT-sensitive tumors, both the sst_5-preferential analog BIM23268 and especially the sst_2 and sst_5 bispecific compound BIM23244, were quite effective in suppressing GH secretion. These data indicate that due to the heterogeneous expression of sst_2 and sst_5 subtypes in GH-secreting adenomas, a bispecific analog, such as BIM-23244 that can activate both receptors, may achieve a better control of GH hypersecretion of GH-producing pituitary tumors than OCT.

Bruns *et al.* (16) and Lewis *et al.* (17) synthesized SOM230, a stable SS analog with a more universal binding profile to sst subtypes. By using alanine scanning technology, essential functional groups of the SS peptide responsible for the high affinity to all five sst subtypes were detected. Incorporation of four synthetic amino acids and two essential amino acids of SS into a stable cyclohexapeptide template resulted in SOM230, a compound that binds with a high affinity to sst_1, sst_2, sst_3, and sst_5 and with low affinity to sst_4. In rats, dogs,

Abbreviations: CD, Control day; HPRT, hypoxanthine-guanine phosphoribosyl transferase; OCT, octreotide; oGTT, oral glucose tolerance test; PRL, prolactin; SS, somatostatin; sst, SS receptor.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

and monkeys, SOM230 potently and dose-dependently decreases GH and IGF-I levels. Only minimal desensitization of the suppressing effect of SOM230 on IGF-I levels under physiological conditions was observed, which is in contrast to what has been seen in rodents with the effect of OCT. Additional characteristics of SOM230 include a favorable terminal elimination half-life of 27 h in humans as well as the preliminary evidence that glucose levels in rats and dogs remain normal during long-term administration of the compound.

Here, we present the detailed analysis of the first single-dose, proof-of-concept study with SOM230 in acromegalic patients. A double-blind, randomized, crossover study was performed to compare the *in vivo* effects of a single dose of SOM230 to OCT on GH release, to assess its safety and tolerability in 12 patients with active acromegaly.

Patients and Methods

Patients

Twelve patients with active acromegaly were recruited at the Erasmus Medical Center in Rotterdam, The Netherlands. All subjects had biochemically active disease, with a mean serum GH concentration greater than 5 μg/liter during a 5-h profile and elevated circulating IGF-I levels (age and sex adjusted). GH concentration failed to suppress less than 1 μg/liter after a 2-h 75-g oral glucose tolerance test (oGTT). Table 1 shows the biochemical characteristics of the 12 patients. One insulin-treated patient with type 2 diabetes did not undergo an oGTT. Seven patients had been treated before (see below). In those patients who have been medically treated previously, a washout period after the last dose of medication had to be at least 1 month, 1 wk, 4 months, and 1 month for dopamine agonists, sc formulations of OCT, depot formulations of long-acting somatostatin analogs, and GH receptor antagonists, respectively. One patient had been previously treated by surgery, medical treatment, and irradiation. One patient was treated only with surgery. Two patients had only been medically treated, and three patients were treated with surgery and medical treatment. Five patients were newly diagnosed. Patients with compression of the optic chiasm causing any visual field defect or those requiring surgical intervention for relief of any sign or symptom possibly associated with tumor compression were excluded. The study was approved by the local ethical committee of the Erasmus Medical Center, and all patients gave written informed consent.

Treatment protocol

Patients were hospitalized on the control day (CD) for 24 h for the assessment of baseline efficacy parameters. On study d 1, 8, and 15, each patient received at 0900 h a single sc injection of 100 μg OCT, 100 μg SOM230, or 250 μg SOM230 in a randomized, double-blinded, crossover fashion with a minimum 6 d of washout between drug treatments. All patients received standarized meals, served at 0830, 1230, and 1730 h. Blood samples, withdrawn through an indwelling venous catheter placed in the forearm, for the assessment of GH and PRL concentrations, were collected at 30 min, 1 min before, and every hour for 24 h after drug administration. This procedure was repeated on all study days. Furthermore, blood samples for glucose and insulin assessments were collected 30 min, 1 min before, and every half-hour for 2 h after lunch. Blood specimens were centrifuged, and the plasma was frozen at −20 C until it was assayed.

Safety assessments included vital signs (pulse rate, blood pressure, and temperature); electrocardiograms; biochemistry; hematology; and urinalysis.

Assays

GH (micrograms/liter), PRL (micrograms/liter), and insulin (milliunits/liter; 1 mU/liter = 7.175 pmol/liter) levels were determined by use of a nonisotopic, automatic chemiluminescence immunoassay system [Immulite; Diagnostic Products Corp., Los Angeles, CA]. The intra- and interassay coefficients of variation for GH, PRL, and insulin were 6.0, 5.7, 4.4, and 6.2, 6.4, 5.9%, respectively. Glucose (millimoles/liter; 1 mmol/liter = 18.015 mg/dl) was measured with an automatic hexokinase method (Roche, Almere, The Netherlands). Serum IGF-I (micrograms/liter) was determined with a commercially available nonextraction immunoradiometric assay (Diagnostic Systems Laboratories, Inc., Webster, TX) (intra- and interassay coefficients of variation, 3.9 and 4.2%, respectively).

In vitro studies

Two patients underwent transphenoidal surgery 3 months before they entered the study. Adenomatous tissue was collected during operation, and, subsequently, pituitary adenoma cells were isolated as described previously (18). The viability of the resulting cell suspension, as determined by trypan blue dye exclusion, was greater than 95%. The cells were cultured at a density of $0.5–1 \times 10^5$ cells/dish·1 ml in multiwell plates (Corning Costar, Cambridge, MA). The culture medium was Eagle's MEM with Earle's salts supplemented with a 1-fold excess of nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone, and 10% fetal calf serum (Invitrogen, Breda, The Netherlands). Media and supplements were obtained from Life Technologies, Inc. Bio-Cult Europe (Invitrogen). The cells were allowed to attach for at least 3 d before a 72-h incubation with SS (Sigma, St. Louis, MO), OCT, or SOM230 (both donated by Novartis, Basel, Switzerland) in 1 ml complete culture medium performed with the attached cells, using four dishes for every treatment group. These pituitary cell cultures are primary cultures that were plated immediately after the isolation, and they were not

TABLE 1. Patients' characteristics on study entry

Patient	Sex	Age (yr)	GH (μg/liter)[a]	PRL (μg/liter)[a]	IGF-I (μg/liter)[b]	GH after oGTT (μg/liter)[c]
1	F	43	13.6	13.8	1981	10.1
2	M	34	7.4	18.1	1301	8.3
3	F	55	31.6	3.4	1385	29.1
4	M	48	26.5	7.8	1515	ND
5	F	36	31.1	7.2	941	21.2
6	M	35	48.1	19.1	1729	29.0
7	M	38	19.5	20.9	2027	16.9
8	M	33	11.0	10.3	1920	11.0
9	F	79	19.3	0.7	1148	24.6
10	M	67	6.9	17.7	849	5.5
11	F	52	5.8	9.8	773	2.5
12	F	40	57.4	6.7	2180	75.0

F, Female; M, male; ND, not determined.

[a] Mean fasting GH and PRL values, collected in a 5-h time period. Normal values: GH, <5 μg/liter; PRL, <25 μg/liter (men) or <44 μg/liter (women).

[b] Range in healthy population: 107–497 μg/liter.

[c] Serum GH level after a 2-h 75-g OGTT (normal nadir GH, <1 μg/liter after 2 h).

passaged before the incubation studies were performed. The results of each experiment were expressed as nanograms per dish and compared with control untreated dishes.

Quantitative RT-PCR

Quantitative RT-PCR was performed as described previously (19). Briefly, poly A^+ mRNA was isolated during Dynabeads Oligo $(dT)_{25}$ (Dynal AS, Oslo, Norway) from adenoma cell pellets containing $0.5–1 \times 10^6$ cells/sample. cDNA was synthesized using the poly A^+ mRNA captured on the Dynabeads Oligo $(dT)_{25}$ as a solid-phase and first primer. To quantify sst_2 and sst_5 mRNAs, a quantitative RT-PCR was performed by TaqMan Gold nuclease assay (Perkin-Elmer Corp., Foster City, CA) and the ABI PRISM 7700 sequence detection system (Perkin-Elmer Corp.) for real-time amplification, according to the manufacturer's instructions. The specific primer sequences (Biosource, Nivelles, Belgium) that were used include: sst_2 forward, 5′-TCGGCCAAGTGGAGGAGAC-3′; sst_2 reverse, 5′-AGAGACTCCCCACACAGCCA-3′; sst_5 forward, 5′-CATCCTCTCCTACGCCAACAG-3′; sst_5 reverse, 5′-GGAAGCTCTGGCGGAAGTT-3′; hypoxanthine-guanine phosphoribosyl transferase (HPRT; as a control) forward, 5′-TGCTTTCCTTGGTCAGGCAGTAT-3′; HPRT reverse, 5′-TCAAATCCAACAAAGTCTGGCTTATATC-3′. The probe sequences that were used included: sst_2, 5′-FAM-CCGGACGGCCAAGATGATCACC-TAMRA-3′; sst_5, 5′-FAM-CCCGTCCTCTACGGCTTCCTCTCTGA-TAMRA-3′; HPRT, 5′-FAM-CAAGCTTGCGACCTTGACCATCTTTGGA-TAMRA-3′. The amount of sst_2 and sst_5 mRNA was determined by means of a standard curve generated in each experiment from known amounts of human genomic DNA. For the determination of the amount of HPRT mRNA, a standard curve was obtained by including dilutions of a pool cDNA known to contain HPRT. The amount of sst_2 and sst_5 mRNA was calculated relative to the amount of HPRT and is given in arbitrary units.

Statistical analysis

The assumption of normality of all *in vivo* data was investigated by use of a Kolmogorov-Smirnov test, in which the null hypothesis that the data represented a random sample from the normal distribution was tested. When this hypothesis was not rejected, a paired Student's *t* test was used for assessing the statistical significance, compared with the CD. The Wilcoxon's signed rank test, a nonparametric analog to the paired *t* test, was used when data did not represent a random sample from normal distribution. Correlation analysis was performed by the use of Spearman's rank correlation test. In the *in vitro* studies, one-way ANOVA was used. When significant overall effects were obtained by this method, comparisons were made using Newman-Keuls multiple comparison test. Data are expressed as mean ± SEM. $P < 0.05$ was considered significant.

Results

Safety and tolerability

Tolerability of OCT and SOM230 was good. Local reactions at the injection site were not observed. Side effects probably related to the study drug were reported in three different patients and were mild (one case of palpitation and sweating after 100 μg SOM230, one case of abdominal discomfort after 100 μg OCT and after 250 μg SOM230). No clinically relevant changes in vital signs, routine chemistry, and urinalysis were observed. Electrocardiogram analyses showed no newly occurring or worsening of known cardiac abnormalities 2 and 24 h after injection with OCT or SOM230.

In vivo studies

Figure 1 depicts the mean circulating 24-h GH concentrations after a single sc injection of 100 μg OCT, 100 μg SOM230, and 250 μg SOM230, compared with CD, for all acromegalic patients investigated. Because all three treatment options appeared to induce their effect on GH secretion predominantly immediately after sc injection, efficacy analysis of OCT and the two dosages of SOM230 was assessed by analysis of the mean GH suppression between 2 and 8 h after sc injection, compared with the same period on the CD. The mean GH levels from 2–8 h after 250 μg SOM230, 100 μg SOM230, and OCT were suppressed by 61 ± 6.7% ($P < 0.0001$), 38 ± 7.7% ($P < 0.001$), and 59 ± 9.2% ($P < 0.0001$), respectively. Furthermore, the 250-μg dosage of SOM230 induced a significantly greater suppressive effect on circulating GH concentrations than the 100-μg dosage of SOM230 ($P < 0.01$). The inhibitory effect of OCT on GH levels did not differ from SOM230 250 μg (P = not significant), whereas, compared with 100 μg SOM230, a stronger suppression of GH concentrations by OCT was found, although this difference failed to reach statistical significance ($P = 0.13$).

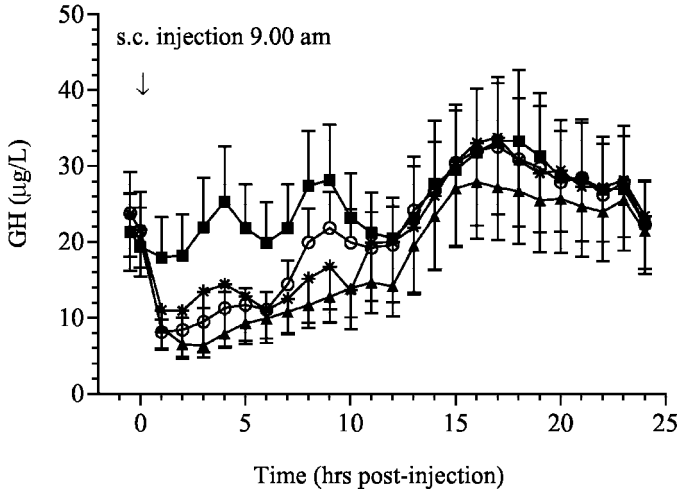

FIG. 1. Twenty-four-hour GH concentration curves on the CD (■—■) and treatment days after sc injection of 100 μg OCT (*—*),250 μg SOM230 (▲—▲), and 100 μg SOM230 (○—○). Data are expressed as mean ± SEM (n = 12).

Analysis of the 12 individual 24-h GH profiles on CD and after administration of the study drugs, revealed three different patterns of response. As illustrated by the 24-h GH profile of patient 6 (Fig. 2A), both OCT and 250 μg SOM230 induced a similar inhibitory effect on circulating GH concentrations [−63%, plasma GH levels 18.9 ± 1.1 μg/liter after OCT ($P < 0.05$) and −65%, plasma GH levels 17.7 ± 1.7 μg/liter after SOM230 ($P < 0.01$), both *vs.* 50.8 ± 4.5 μg/liter on CD]. A comparable suppressive effect on GH levels by 250 μg OCT and SOM230 was observed in a total of eight patients. In this subgroup of eight equal responders, both SOM230 and OCT significantly suppressed GH levels by 65 ± 7% (8.0 ± 2.7 *vs.* 20.4 ± 6.5 μg/liter on CD, $P < 0.05$) and 72 ± 7% (7.5 ± 3 *vs.* 20.4 ± 6.5 μg/liter on CD, $P < 0.05$; OCT *vs.* SOM230, P = not significant), respectively (Fig. 3A).

The second pattern of response to the study drugs, observed in a subgroup of three patients, is illustrated by the 24-h GH profile of patient 12 (Fig. 2B). In this particular patient, no decline in circulating GH concentrations after OCT administration was seen (mean plasma GH level 76.7 ± 7.3 *vs.* 67.4 ± 4.8 μg/liter on CD). However, a significant suppression of serum GH levels after administration of SOM230 was found (−68%; 21.9 ± 2.2 μg/liter *vs.* 67.4 ± 4.8

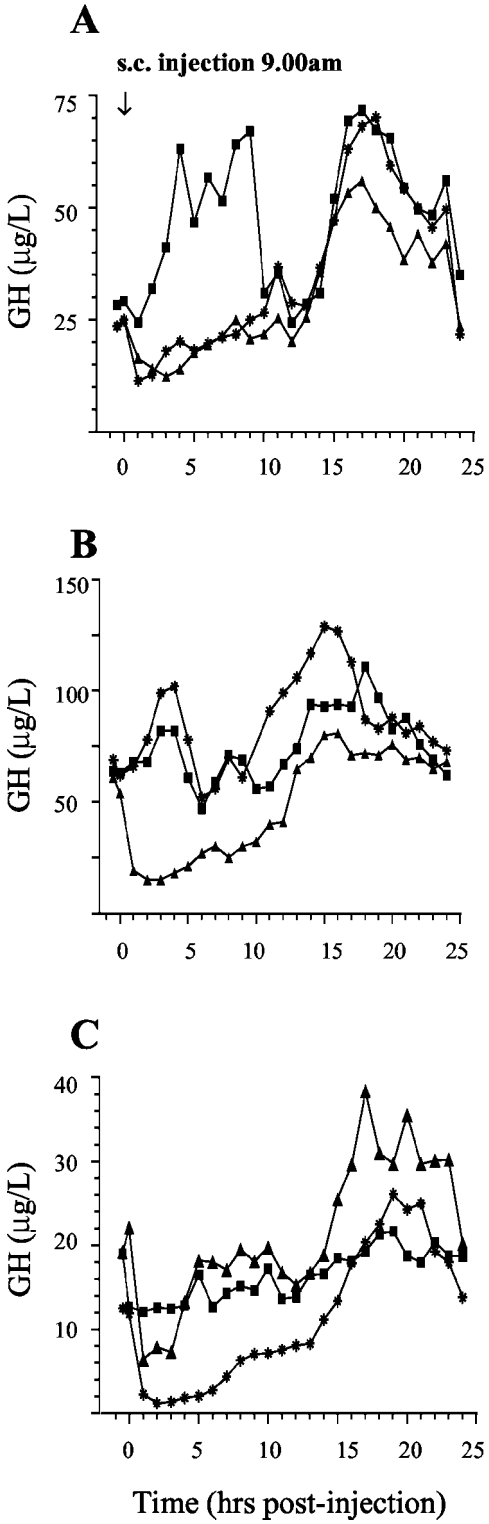

FIG. 2. Twenty-four-hour GH concentration curves of the different response patterns after sc injection of the study drugs, represented by patients 6 (A), 12 (B), and 8 (C). Symbols display CD (■—■), 100 μg OCT (*—*), and 250 μg SOM230 (▲—▲).

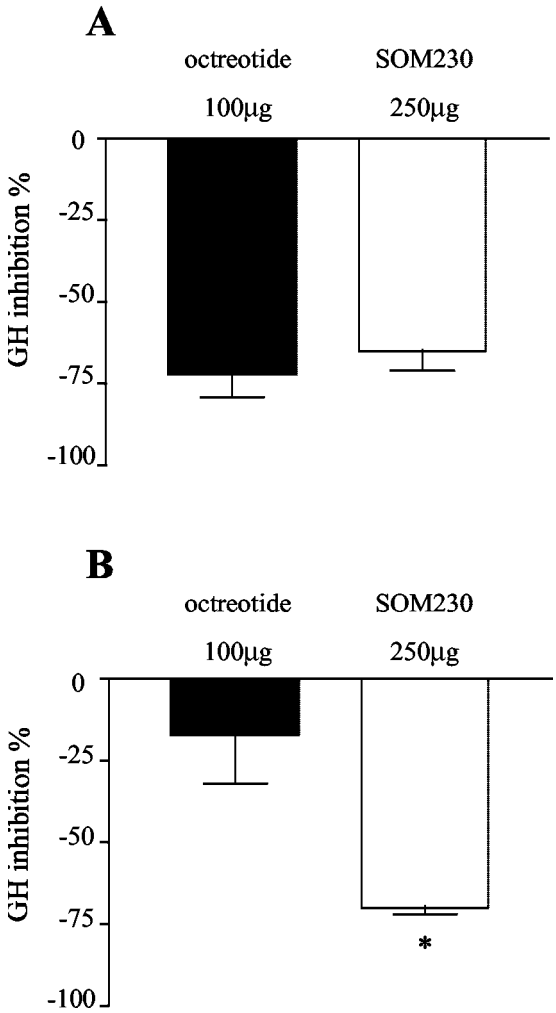

FIG. 3. GH suppression 2–8 h after sc injection. The *bars* represent mean ± SEM percentual GH suppression induced by 100 μg octreotide (■) and 250 μg SOM230 (□), compared with the CD. A, Group showing equal response to OCT and SOM230 (n = 8). B, Group showing higher sensitivity to SOM230 (n = 3; *, $P < 0.05$).

μg/liter on CD, $P < 0.05$; SOM230 *vs.* OCT, $P < 0.01$), and, interestingly, in this particular patient the observed potent suppression by the high-dose 250 μg SOM230 was also achieved by the low-dose 100 μg SOM230 (−64%; 24.4 ± 3.4 μg/liter). As shown in Fig. 3B, the mean suppression of GH levels in these three patients by SOM230 was significantly greater than the suppressive effect by OCT [−70 ± 2%, 9.7 ± 6 μg/liter and −17 ± 15%, 30.9 ± 23 μg/liter, respectively, *vs.* 30.5 ± 19 μg/liter on CD (SOM230 *vs.* CD, $P < 0.01$; SOM230 *vs.* OCT, $P < 0.05$ and OCT *vs.* CD, P = n.s.)].

Patient 8 demonstrated a third observed response pattern (Fig. 2C), which showed a significant inhibition by OCT (−79%; mean GH level, 2.9 ± 0.7 *vs.* 13.8 ± 0.6 μg/liter on CD; $P < 0.01$). SOM230 was not effective during the full 2- to 8-h postinjection time interval to elicit an inhibitory effect on circulating GH concentrations (14.5 ± 1.9 *vs.* 13.8 ± 0.6 μg/liter on CD, P = not significant). However, this patient was not insensitive to SOM230 because a short-lasting suppressive effect of SOM230 was established (−40%; mean GH level 1–3 h after administration, 7.2 ± 0.4 *vs.* 12.4 ± 0.2 μg/liter on CD; $P < 0.001$). Still, in this short period of time, OCT induced a more powerful 87% suppression of GH con-

centrations (1.6 ± 0.3 μg/liter; OCT *vs.* CD, $P < 0.001$; OCT *vs.* SOM230, $P < 0.001$).

PRL levels of all 12 patients were within the normal range [mean of five blood samples, <25 μg/liter (men) or 44 μg/liter (women); Table 1]. In two patients, plasma PRL levels decreased after sc injection of OCT as well as with SOM230 (data not shown). Interestingly, the 24-h circulating plasma curves of GH and PRL levels in one of these patients was highly correlated on CD and at treatment days with OCT and SOM230 ($r_s = 0.77$, 0.99, and 0.95, respectively, all $P < 0.001$), which suggests a mixed GH/PRL-secreting pituitary adenoma that cosecreted both hormones from the same adenoma cell.

Figure 4 shows the mean glucose and insulin concentrations of 11 patients (patient 4 was excluded because he was an insulin-treated patient with type 2 diabetes), starting 3 h after sc administration until 2 h after lunch, compared with the same period of time on CD. Compared with the mean glucose level at 1200 h (3 h after dose) on CD (4.4 ± 0.2 mmol/liter), elevated glucose levels were observed after OCT (6.2 ± 0.3 mmol/liter; $P < 0.05$), 250 μg SOM230 (6.1 ± 0.8 mmol/liter; $P < 0.05$) and 100 μg SOM230 (5.8 ± 0.6 mmol/liter; $P < 0.05$) administration (Fig. 4A). When no study drug was administered, lunch induced a physiological increase in mean glucose levels to a maximum of 6.1 ± 0.3 mmol/liter. Similar postprandial responses were observed on all three treatment days (OCT, 6.8 ± 0.5 mmol/liter; 250 μg SOM230, 7.1 ± 0.6 mmol/liter; and 100 μg SOM230, 6.8 ± 0.4 mmol/liter). The highest plasma glucose levels were 10.8 and 13.1 mmol/liter 5 h after injection of OCT and 250 μg SOM230, respectively, and were both observed in patient 1, who was known to have an impaired glucose tolerance (assessed by oGTT before start of the trial). Overall, there was a trend that OCT and both SOM230 dosages induced a comparable increase in mean glucose levels, that responded equally to a meal at 1230 h, compared with CD.

OCT induced an inhibitory effect on mean plasma insulin levels compared with CD, which sustained until 2 h after lunch (1200–1430 h; $P < 0.05$). Mean insulin levels seemed not to be affected by both SOM230 dosages because also after lunch a similar increase in insulin levels was observed as on CD (Fig. 4B). Patient 12 had severe insulin resistance. On the CD, 30 min after lunch was consumed, a sharp increase in plasma insulin levels to a maximum 348 mU/liter was found. This was even more pronounced after 250 μg SOM230 was administered, when insulin levels rose to a maximum of 903 mU/liter (1 h after lunch). However, the blood glucose concentrations of this patient remained within the range of the other nondiabetic patients (Fig. 4A).

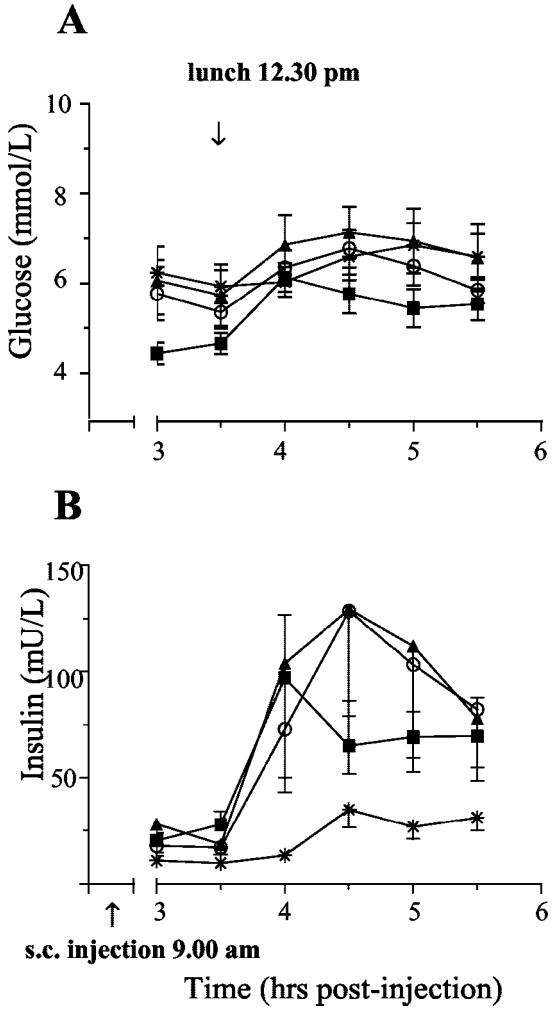

FIG. 4. Mean (±SEM) serum glucose (A) and insulin (B) profiles of 11 patients (one patient was excluded because of insulin-treated type 2 diabetes) during CD (■—■) and on treatment days after sc injection of 100 μg OCT (*—*), 250 μg SOM230 (▲—▲), and 100 μg SOM230 (○—○).

In vitro studies

Apart from the direct effects of OCT and SOM230 on GH release by cultured pituitary tumor cells from two patients, the native peptide SS was also tested. GH production in the control wells from the adenoma cells of patients 6 and 12 after a 72-h incubation amounted to 228 ± 40 and 312 ± 18 ng/dish, respectively. In agreement with the *in vivo* response of patients 6 and 12 (Fig. 2, A and B, respectively), 10 nM SOM230 lowered significantly GH secretion by −32.7 ± 6.8 and −23 ± 6.9% in the primary tumor cell cultures of patients 6 and 12, respectively ($P < 0.05$ in both instances), whereas 10 nM OCT inhibited only the GH secretion in the adenoma cells of patient 6 (−26.1 ± 10.5%; $P < 0.05$; Fig. 5A). SS lowered GH secretion in both primary cultures as well

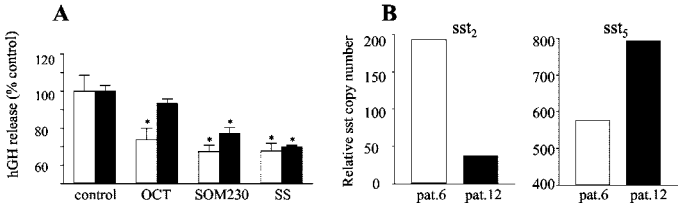

FIG. 5. *In vitro* data of two patients. Percentual inhibition of GH secretion by 10 nM OCT, SOM230, and SS compared with control, after a 72-h incubation in primary cultured pituitary adenoma cells from patients 6 (□) and 12 (■). Data are expressed as mean ± SEM; *, $P < 0.05$ treatment *vs.* control (A). Quantitative analysis of RT-PCR showing the different amount of sst_2 and sst_5 mRNAs in the adenoma tissues of patients 6 (pat.6) and 12 (pat.12), calculated relative to the amount of HPRT and given in arbitrary units (B).

(−32.4 ± 8.5 and −30.1 ± 1.8%, respectively; $P < 0.05$, Fig. 5A). Furthermore, evaluation of the relative mRNA expression levels for sst_2 and sst_5 in both cases revealed an interesting difference. The adenoma cells from patient 6, who responded to all three compounds, had a relatively high expression of sst_2 (193 copies/HPRT) and a relatively low expression of sst_5 (577 copies/HPRT). Compared with mRNA expression levels in the adenoma cells of patient 12, which were responsive only to SOM230 and SS treatment, an opposite mRNA expression pattern was found (Fig. 5B). The pituitary adenoma of patient 12 contained relatively high mRNA expression levels for sst_5 (793 copies/HPRT) and approximately 5-fold lower sst_2 mRNA expression levels than those of patient 6 (37 copies/HPRT). The adenoma was *in vivo* and *in vitro* not responsive to OCT but demonstrated significant sensitivity to both dosages of SOM230 *in vivo* and to SOM230 *in vitro*. This suggests the involvement of sst_5 subtype in the GH-release inhibitory effect in this particular case.

Discussion

In the present study, the recently developed SS analog SOM230, exhibiting a universal binding profile that was demonstrated to effectively suppress GH levels in normal monkeys and rodents (16), was administered for the first time in acromegalic patients to assess its efficacy in comparison with OCT. The acute effects of a single dose of 250 μg SOM230 and 100 μg OCT on circulating GH concentrations demonstrated three patterns of response in the 12 patients investigated. In eight patients, both SS analogs were equally effective in lowering GH levels. This suggests that in these patients the sst_2 subtype is the major receptor on the pituitary adenoma, which is responsible for mediating these inhibitory effects. It is well known that sst_2 especially is involved in the inhibitory actions of SS and SS analogs on hormone secretion, both in primary cultured human fetal pituitary cells and GH-secreting pituitary adenoma cells as well (12, 20). The relative amount of mRNA expression levels of this receptor subtype was positively correlated with the sensitivity to OCT treatment *in vitro* (21). The *in vitro* data of patient 6 illustrate in this group of equal responders that the relatively high mRNA level for sst_2 combined with the good affinity of both SOM230 and OCT account for the suppressive effects of both drugs on GH secretion in this group of patients. SOM230 has a 2.5 times lower affinity to sst_2 than OCT, which explains the similar effect of 250 μg SOM230 and 100 μg OCT in this category of acromegalic patients.

The second pattern of response, illustrated by patient 12 in which SOM230 is far more efficacious, compared with OCT in suppressing GH levels, was observed in three patients. A pivotal role for sst_5 in mediating suppression of GH release is probable. The *in vitro* data of patient 12 show relatively low mRNA expression levels for sst_2 and higher expression levels for sst_5. The IC_{50} for sst_5 of SOM230 is 0.16 nmol/liter, and that of OCT is 40 times higher (6.3 nmol/liter), pointing to the higher affinity of SOM230 for the sst_5 subtype. These observations, together with the the *in vitro* significant inhibition by the native SS on the primary culture of adenoma cells, suggest that both SS and SOM230 exert their potent effects in this particular tumor via sst_5 subtype. So far, the role of sst_5 in mediating GH release was investigated only in studies with primary cultures of pituitary adenoma cells obtained from acromegalic patients (20, 21). Saveanu *et al.* (15) found a 30-fold higher expression of sst_5 mRNA, compared with sst_2 mRNA, in four adenomas poorly responsive to OCT. The addition of the sst_5-specific analog BIM23268 to the medium achieved a maximal GH suppression. This suggests a rescue through sst_5 when tumors are only partially sensitive to OCT (15). SOM230 induced a 3-fold stronger inhibition than OCT on GH release by cultured rat pituitary cells and a pronounced inhibition of plasma IGF-I levels in rodents after 18 wk of treatment, which again is suggestive for sst_5 involvement (16).

We present the first clinical evidence that the sst_5 subtype may indeed play an essential role in mediating the *in vivo* suppressive actions by SOM230 on GH concentrations in three acromegalic patients, which were (partially) unresponsive to OCT. Because SOM230 is able to lower GH levels in both subgroups of patients, coupled with sst_2 and sst_5 subtype physiology, respectively, this novel SS analog has a clear advantage over OCT and might increase the number of patients who can be biochemically controlled during long-term medical treatment. Furthermore, in patient 12, 100 and 250 μg SOM230 suppressed GH levels equally. In this particular case, increasing the SOM230 dosage by a factor 2.5 did not result in a further increase in GH inhibition. This phenomenon is already known for patients who are sensitive to OCT treatment: a similar GH suppression is found on sc injections with OCT dosages in the range of 100-1500 μg/d (22, 23). This could indicate that the density of the predominantly expressed sst determines the response to a SS analog: in GH-secreting adenomas expressing sst_2 in high density, OCT is able to suppress GH levels significantly. However, if sst_2 is almost not expressed on the pituitary adenoma, sst_5 mediates the GH-suppressive effects of SS and SS analogs. The dose-response curves of OCT and SOM230 seem to reach the plateau at low levels when high densities of sst_2 and sst_5, respectively, are expressed. *In vivo* and *in vitro* data from this trial emphasize that the inhibitory effects on GH release by SS and its analogs are primarily mediated via sst_2, as seen in the group of eight equal responders to OCT and SOM230. However, when sst_2 over sst_5 mRNA levels are being expressed below a certain threshold, as in patient 12, a suppressive action on GH concentrations via sst_5 receptors becomes visible. In addition, heterodimeric effects of different sst subtypes are suggested to play a role in receptor physiology (24, 25), and, as discussed previously, the BIM23244 bispecific sst_{2+5} analog has already shown to be more active than the combination of a sst_2-specific analog combined with a sst_5 specific analog on GH release (15, 26), indicating that a heterodimeric effect by SOM230 on sst_2 and sst_5 subtypes cannot be ruled out.

The third response was observed in one patient, who only transiently responded to SOM230, whereas OCT was far more efficacious in lowering GH levels. The most likely explanation is the presence of a relatively high sst_2 and a low sst_5 mRNA expression level, resulting in a high sensitivity for OCT. Whether higher dosages of SOM230 would indeed

induce similar lowering actions on GH concentrations as seen by OCT, remains uncertain.

Because SS and its analogs inhibit the secretion of insulin, impaired postprandial glucose tolerance was observed after the acute administration of OCT (27). Similar elevations of glucose concentrations were observed after SOM230. However, the elevated glucose levels seem not be caused by an inhibitory action on insulin release because after SOM230 administration at 0900 h, an almost identical insulin response was observed after lunch as on the CD. At present, the mechanism of this transient increase in glucose levels remains uncertain. Several studies support a role for sst_5 to control insulin secretion in rats, mice, and humans, whereas sst_2 mediates glucagon secretion from the pancreatic α-cells (28–31). On the basis of the SOM230 and OCT affinity profiles for sst_2 and sst_5, it seems unlikely that OCT, binding 40-fold less to sst_5 compared with SOM230, would exert such a strong and long-lasting insulin inhibition via sst_5 subtype, whereas SOM230 treatment resulted in barely any inhibition. Therefore, these opposed effects of OCT and SOM230 on insulin levels suggest a pivotal role for sst_2 subtype in regulating human insulin secretion. In cynomolgus monkeys, insulin, glucagon, and glucose levels remained unchanged during 7 d of high-dose infusion with SOM230. Furthermore, during an 18-wk treatment with pharmacological doses of SOM230, plasma glucose levels were not changed, indicating that SOM230 is well tolerated in rats and monkeys with regard to glucose homeostasis (16, 32).

The promising pharmokinetic properties of SOM230 found *in vivo* in rats, accounting for a terminal elimination half-life of 27 h compared with 2 h for OCT (16), did not result in a longer duration of action of SOM230 than that of OCT. Probably, serum SOM230 concentrations drop sooner below a certain therapeutical level, leading to a duration of action on GH levels comparable with that of OCT treatment.

In conclusion, our data suggest that SOM230 has the potency to increase the number of acromegalic patients who can be biochemically controlled during long-term medical treatment because of its additional suppressive effects on GH secretion via sst_5. However, the subtype sst_2 seems to be the dominant receptor in controlling hypersecretion in acromegaly. No serious side effects occurred during SOM230 treatment. The subtle increase in glucose levels after SOM230 injection needs additional attention and cannot be explained by sst_5- or sst_2-mediated action on insulin secretion. Future studies will also address the question of whether SOM230 can control pituitary adenoma size in acromegaly better than OCT (9). Besides sst_2 and sst_5, sst_1 and sst_3 also seem to be involved in cell proliferation and the induction of apoptosis (33–36). This suggests that the universal SS analog SOM230, with good affinity for both sst_1 and sst_3, might have possible antiproliferative and tumor size reducing effects as well.

Acknowledgments

Received July 7, 2003. Accepted October 19, 2003.

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References

1. **Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R** 1973 Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77–79
2. **Lamberts SW, Uitterlinden P, Verschoor L, van Dongen KJ, del Pozo E** 1985 Long-term treatment of acromegaly with the somatostatin analogue SMS 201–995. N Engl J Med 313:1576–1580
3. **Lamberts SW, Oosterom R, Neufeld M, del Pozo E** 1985 The somatostatin analog SMS 201–995 induces long-acting inhibition of growth hormone secretion without rebound hypersecretion in acromegalic patients. J Clin Endocrinol Metab 60:1161–1165
4. **Newman CB, Melmed S, George A, Torigian D, Duhaney M, Snyder P, Young W, Klibanski A, Molitch ME, Gagel R, Sheeler L, Cook D, Malarkey W, Jackson I, Vance ML, Barkan A, Frohman L, Kleinberg DL** 1998 Octreotide as primary therapy for acromegaly. J Clin Endocrinol Metab 83:3034–3040
5. **Freda PU** 2002 Somatostatin analogs in acromegaly. J Clin Endocrinol Metab 87:3013–3018
6. **Caron P, Beckers A, Cullen DR, Goth MI, Gutt B, Laurberg P, Pico AM, Valimaki M, Zgliczynski W** 2002 Efficacy of the new long-acting formulation of lanreotide (lanreotide Autogel) in the management of acromegaly. J Clin Endocrinol Metab 87:99–104
7. **Chanson P, Boerlin V, Ajzenberg C, Bachelot Y, Benito P, Bringer J, Caron P, Charbonnel B, Cortet C, Delemer B, Escobar-Jimenez F, Foubert L, Gaztambide S, Jockenhoevel F, Kuhn JM, Leclere J, Lorcy Y, Perlemuter L, Prestele H, Roger P, Rohmer V, Santen R, Sassolas G, Scherbaum WA, Schopohl J, Torres E, Varela C, Villamil F, Webb SM** 2000 Comparison of octreotide acetate LAR and lanreotide SR in patients with acromegaly. Clin Endocrinol (Oxf) 53:577–586
8. **Lancranjan I, Atkinson AB** 1999 Results of a European multicentre study with Sandostatin LAR in acromegalic patients. Sandostatin LAR Group. Pituitary 1:105–114
9. **Bevan JS, Atkin SL, Atkinson AB, Bouloux PM, Hanna F, Harris PE, James RA, McConnell M, Roberts GA, Scanlon MF, Stewart PM, Teasdale E, Turner HE, Wass JA, Wardlaw JM** 2002 Primary medical therapy for acromegaly: an open, prospective, multicenter study of the effects of subcutaneous and intramuscular slow-release octreotide on growth hormone, insulin-like growth factor-I, and tumor size. J Clin Endocrinol Metab 87:4554–4563
10. **Colao A, Ferone D, Marzullo P, Cappabianca P, Cirillo S, Boerlin V, Lancranjan I, Lombardi G** 2001 Long-term effects of depot long-acting somatostatin analog octreotide on hormone levels and tumor mass in acromegaly. J Clin Endocrinol Metab 86:2779–2786
11. **Hofland LJ, Lamberts SW** 2003 The pathophysiological consequences of somatostatin receptor internalization and resistance. Endocr Rev 24:28–47
12. **Shimon I, Taylor JE, Dong JZ, Bitonte RA, Kim S, Morgan B, Coy DH, Culler MD, Melmed S** 1997 Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation. J Clin Invest 99: 789–798
13. **Panetta R, Patel YC** 1995 Expression of mRNA for all five human somatostatin receptors (hSSTR1–5) in pituitary tumors. Life Sci 56:333–342
14. **Schaer JC, Waser B, Mengod G, Reubi JC** 1997 Somatostatin receptor subtypes sst1, sst2, sst3 and sst5 expression in human pituitary, gastroentero-pancreatic and mammary tumors: comparison of mRNA analysis with receptor autoradiography. Int J Cancer 70:530–537
15. **Saveanu A, Gunz G, Dufour H, Caron P, Fina F, Ouafik L, Culler MD, Moreau JP, Enjalbert A, Jaquet P** 2001 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. J Clin Endocrinol Metab 86:140–145
16. **Bruns C, Lewis I, Briner U, Meno-Tetang G, Weckbecker G** 2002 SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile. Eur J Endocrinol 146:707–716
17. **Lewis I, Bauer W, Albert R, Chandramouli N, Pless J, Weckbecker G, Bruns C** 2003 A novel somatostatin mimic with broad somatotropin release inhibitory factor receptor binding and superior therapeutic potential. J Med Chem 46: 2334–2344
18. **Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SW** 1984 Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 7:307–311
19. **Ferone D, Pivonello R, Van Hagen PM, Dalm VA, Lichtenauer-Kaligis EG, Waaijers M, Van Koetsveld PM, Mooy DM, Colao A, Minuto F, Lamberts SW, Hofland LJ** 2002 Quantitative and functional expression of somatostatin receptor subtypes in human thymocytes. Am J Physiol Endocrinol Metab 283:E1056-E1066
20. **Shimon I, Yan X, Taylor JE, Weiss MH, Culler MD, Melmed S** 1997 Somatostatin receptor (SSTR) subtype-selective analogues differentially suppress *in vitro* growth hormone and prolactin in human pituitary adenomas. Novel potential therapy for functional pituitary tumors. J Clin Invest 100:2386–2392
21. **Jaquet P, Saveanu A, Gunz G, Fina F, Zamora AJ, Grino M, Culler MD, Moreau JP, Enjalbert A, Ouafik LH** 2000 Human somatostatin receptor sub-

types in acromegaly: distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes. J Clin Endocrinol Metab 85:781–792
22. **Ezzat S, Redelmeier DA, Gnehm M, Harris AG** 1995 A prospective multicenter octreotide dose response study in the treatment of acromegaly. J Endocrinol Invest 18:364–369
23. **Vance ML, Harris AG** 1991 Long-term treatment of 189 acromegalic patients with the somatostatin analog octreotide. Results of the International Multicenter Acromegaly Study Group. Arch Intern Med 151:1573–1578
24. **Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC, Patel YC** 2000 Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. J Biol Chem 275:7862–7869
25. **Pfeiffer M, Koch T, Schroder H, Klutzny M, Kirscht S, Kreienkamp HJ, Hollt V, Schulz S** 2001 Homo- and heterodimerization of somatostatin receptor subtypes. Inactivation of sst(3) receptor function by heterodimerization with sst(2A). J Biol Chem 276:14027–14036
26. **Shimon I** 2003 Somatostatin receptors in pituitary and development of somatostatin receptor subtype-selective analogs. Endocrine 20:265–270
27. **Lamberts SW, Uitterlinden P, del Pozo E** 1987 SMS 201–995 induces a continuous decline in circulating growth hormone and somatomedin-C levels during therapy of acromegalic patients for over two years. J Clin Endocrinol Metab 65:703–710
28. **Strowski MZ, Parmar RM, Blake AD, Schaeffer JM** 2000 Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an *in vitro* study of pancreatic islets from somatostatin receptor 2 knockout mice. Endocrinology 141:111–117
29. **Strowski MZ, Kohler M, Chen HY, Trumbauer ME, Li Z, Szalkowski D, Gopal-Truter S, Fisher JK, Schaeffer JM, Blake AD, Zhang BB, Wilkinson HA** 2003 Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. Mol Endocrinol 17:93–106
30. **Mitra SW, Mezey E, Hunyady B, Chamberlain L, Hayes E, Foor F, Wang Y, Schonbrunn A, Schaeffer JM** 1999 Colocalization of somatostatin receptor sst5 and insulin in rat pancreatic beta-cells. Endocrinology 140:3790–3796
31. **Kumar U, Sasi R, Suresh S, Patel A, Thangaraju M, Metrakos P, Patel SC, Patel YC** 1999 Subtype-selective expression of the five somatostatin receptors (hSSTR1–5) in human pancreatic islet cells: a quantitative double-label immunohistochemical analysis. Diabetes 48:77–85
32. **Weckbecker G, Briner U, Lewis I, Bruns C** 2002 SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. Endocrinology 143: 4123–4130
33. **Zatelli MC, Tagliati F, Piccin D, Taylor JE, Culler MD, Bondanelli M, degli Uberti EC** 2002 Somatostatin receptor subtype 1-selective activation reduces cell growth and calcitonin secretion in a human medullary thyroid carcinoma cell line. Biochem Biophys Res Commun 297:828–834
34. **Reisine T, Bell GI** 1995 Molecular biology of somatostatin receptors. Endocr Rev 16:427–442
35. **Benali N, Ferjoux G, Puente E, Buscail L, Susini C** 2000 Somatostatin receptors. Digestion 62:27–32
36. **Ferone D, van Hagen MP, Kwekkeboom DJ, van Koetsveld PM, Mooy DM, Lichtenauer-Kaligis E, Schonbrunn A, Colao A, Lamberts SW, Hofland LJ** 2000 Somatostatin receptor subtypes in human thymoma and inhibition of cell proliferation by octreotide *in vitro*. J Clin Endocrinol Metab 85:1719–1726

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