# In Vitro Head-to-Head Comparison Between Octreotide and Pasireotide in GH-Secreting Pituitary Adenomas

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**Context:** First-generation somatostatin analogs (SSAs), such as octreotide (OCT), are the first line medical therapy for acromegaly. Pasireotide (PAS), a newly developed SSA, has shown promising results in the treatment of acromegaly.

**Objective:** To compare the antisecretory effect of OCT and PAS in primary cultures of growth hormone (GH)–secreting pituitary adenomas (GH-omas). To correlate responses with the adenoma somatostatin receptor (SSTR) profile.

**Design:** The effect of OCT and PAS on GH (and PRL) secretion was tested in 33 GH-oma cultures. SSTR expression was evaluated in adenoma samples.

**Setting and Patients:** Patients with acromegaly referred to the Erasmus Medical Center (Rotterdam, The Netherlands).

**Interventions:** OCT and PAS treatment for 72 hours (10 nM).

**Main Outcome Measures:** GH (and PRL) concentrations in cell culture media. SSTR expression in adenoma samples.

**Results:** The overall effect of OCT (−36.8%) and PAS (−37.1%) on GH secretion was superimposable. We identified three adenoma groups: PAS+ (PAS more effective than OCT), n = 6; PAS = OCT, n = 22; and OCT+ (OCT more effective than PAS), n = 5. PAS+ adenomas showed lower somatostatin receptor subtype $(sst)_2$ messenger RNA (mRNA) and $sst_2/sst_5$ mRNA ratio, compared with the other groups ($P < 0.05$). PAS inhibited PRL hypersecretion more than OCT ($P < 0.01$).

**Conclusions:** Overall, OCT and PAS equally reduced GH secretion *in vitro*. Adenomas with lower $sst_2$ mRNA expression and lower $sst_2/sst_5$ mRNA ratio were better responders to PAS compared with OCT. SSTR evaluation in GH-omas may become a tool for tailored SSA treatment in acromegaly. **(*J Clin Endocrinol Metab* 102: 2009–2018, 2017)**

Growth hormone (GH)–secreting pituitary adenomas represent the most common cause of acromegaly, a severe systemic condition characterized by GH hypersecretion and elevated circulating insulin-like growth factor (IGF)-I levels, which may result in a significant increase in morbidity and mortality (1). Therefore, the main goal of acromegaly

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Abbreviations: GH, growth hormone; hprt, hypoxanthine phosphoribosyltransferase; IGF, insulin-like growth factor; IRS, immunoreactivity score; LAN, lanreotide; mRNA, messenger RNA; OCT, octreotide; PAS, pasireotide; Peg, pegvisomant; SSA, somatostatin analog; sst, somatostatin receptor subtype; SSTR, somatostatin receptor; ULNR, upper limit of normality range.

treatment is the control of GH and IGF-I levels, which can restore normal life expectancy in cured or biochemically controlled patients (2). Based on the well-established high expression of somatostatin receptors (SSTRs) on the GH-secreting adenoma cell membrane, long-acting formulations of the "classically" available somatostatin analogs (SSAs), octreotide (OCT) and lanreotide (LAN), represent nowadays the first-line medical treatment in acromegaly (3, 4).

Furthermore, it is well known that both OCT and LAN, differently from their endogenous counterpart somatostatin (SRIF-14), show a high preferential binding affinity (in the subnanomolar range) for somatostatin receptor subtype $(sst)_2$ and weak–moderate affinity for $sst_3$ and $sst_5$ (5, 6). Moreover, a number of studies have already demonstrated that both $sst_5$ and, in particular, $sst_2$ are highly expressed in GH-secreting adenomas (7, 8). Therefore, $sst_2$ overexpression represents the main pathophysiological rationale for OCT/LAN therapy in patients with acromegaly. In this light, a number of studies have already demonstrated a strong direct positive correlation between OCT/LAN efficacy and $sst_2$ expression [both at the messenger RNA (mRNA) and protein levels] (9–11).

However, differently from initial clinical trials, which were affected by selection bias, updated clinical observations and results from registries, as well as from a recent meta-analysis (12), have shown that medical treatment with these SSAs results in the normalization of GH or IGF-I levels in ~50% of patients with acromegaly. Moreover, a consistent percentage of patients (~30%) seems to be poor responders or even completely resistant to long-term SSA treatment (13).

These findings have pushed researchers to develop novel SSAs with different characteristics compared with the currently available ones, with the main aim to generate compounds with a more universal binding profile for SSTRs, similar to that of endogenous SRIF (the latter not used in clinical practice due to its very short half-life). To our knowledge, among a number of novel compounds tested *in vitro* and described in the recent literature (*e.g.*, somatoprim, dopastatins, KE108) (14–16), pasireotide (PAS) is currently the only SSTR pan-ligand that has been approved for clinical use not only in Cushing disease, but also in acromegaly by the Food and Drug Administration (Novartis media release, December 2014) and the European Medicines Agency. Interestingly, a very recent head-to-head superiority study, comparing the efficacy of OCT and PAS in the treatment of patients with naive acromegaly, has demonstrated that the effect of the two drugs in the reduction of GH levels was superimposable, whereas PAS was more effective in lowering circulating IGF-I levels (17).

PAS is a stable cyclohexapeptide that shows high affinity for multiple SSTRs ($sst_5 > sst_2 > sst_3 > sst_1$) (18). However, despite the initial search for a compound able to closely mimick native SRIF-14, recent studies have demonstrated that PAS shows different functional properties compared with both SRIF-14 and OCT when binding to SSTRs, in particular to $sst_2$. In transfected human cell lines and in rat cell lines endogenously expressing SSTRs, PAS treatment results in a significantly lower $sst_2$ internalization and less $\beta$-arrestin recruitment and leads to a "biased" activation of a number of second messenger pathways (lower activation of the extracellular signal-regulated kinase pathway, no increase of intracellular $Ca^{2+}$, and a slightly less potent cyclic adenosine monophosphate inhibition), compared with SRIF-14 and OCT (19–21) (Supplemental Table 1).

Based on these findings and preclinical studies in Cushing disease (22), PAS is mainly considered a biased agonist for $sst_2$, and its main effects have been related to the activation of other SSTRs than $sst_2$ (in particular $sst_5$).

However, previous studies carried out in a small number of primary cultures of GH-secreting adenomas (<10 samples) have shown that the efficacy of PAS in lowering GH levels *in vitro* is directly correlated with $sst_2$ mRNA expression (but not with $sst_5$) (23) and that the effects of PAS and OCT are almost superimposable in a group of adenoma samples that mainly express $sst_2$ mRNA (24).

Therefore, the correlation between the antisecretory effect of PAS and SSTR profile in GH-secreting adenomas still needs to be better clarified.

As such, the main aims of our study were: (1) to compare the direct *in vitro* antisecretory effect of OCT and PAS on GH secretion (and, when present, on PRL secretion) in a large number of primary cultures of GH-secreting pituitary adenomas (n = 33), and (2) to correlate these data with the adenoma SSTR expression profile at both mRNA and protein levels. Moreover, based on the analysis of membrane receptor profile, we aimed also to identify the presence of peculiar GH-secreting adenoma subpopulations in which the effect of one of the two compounds could be predominant.

## Patients and Methods

### Patients, tumors, and assays

Pituitary tumor samples were obtained by transsphenoidal surgery from 33 patients with acromegaly (19 male, 14 female; median age, 42 years; range, 16 to 65 years).

Diagnosis of acromegaly was primarily based on clinical features, biochemical evidence of GH hypersecretion (lack of suppression of GH to <1 μg/L after a 2-hour oral glucose tolerance test), and IGF-I levels above the age-adjusted upper limit of normality range (ULNR), as well as the identification of a pituitary adenoma by magnetic resonance imaging.

Most patients, 24 out of 32 (75%), were harboring a macroadenoma at baseline magnetic resonance imaging evaluation. Information about tumor size at time of diagnosis was not available in one patient.

Inclusion criteria of the study were: (1) availability of enough viable cells to establish a primary culture; (2) adequate cell number to test in the same experiment, at least in triplicate, the antisecretory effect of 72-hour OCT and PAS treatment (vs control); and (3) enough cells to perform SSTR mRNA evaluation. Exclusion criteria were: (1) available tumor samples from a patient who underwent radiotherapy before adenomectomy, and (2) no possibility to evaluate SSTR mRNA expression. No other exclusion criteria, based on patient or tumor characteristics, were applied in the present study. The time frame of tissue and data collection was 2003 to 2014.

Partial data from four out of 33 patients have been already described in a previous paper from our group, in which the comparison between OCT and PAS was evaluated in a very small series of primary cell cultures (23). For all samples included in the study (n = 33), directly after obtaining the tissue, a piece was used for cell culture, and RNA isolation and following mRNA analysis were carried out from freshly isolated cell pellets. Moreover, we were able to collect paraffin-embedded tissues from 24 adenoma samples to investigate $sst_2$ and $sst_5$ expression at the protein level by immunohistochemistry. In line with the well-established concept that a significant proportion of GH-secreting pituitary adenomas coexpress PRL (25), we observed cosecretion of GH and PRL in 33% (11 of 33) of our cultures.

Approval from the Medical Ethical Committee of the Erasmus University Medical Center and informed consent to use the tumor tissues for research purposes were obtained.

Human GH and PRL concentrations from cell culture media were determined by use of a nonisotopic, automatic chemiluminescence immunoassay system (Immulite; Diagnostic Products, Los Angeles, CA). Intra-assay and interassay coefficients of variation for GH and PRL were 6.0% and 5.7% and 6.2% and 6.4%, respectively. Not all parameters were available for each patient.

## Cell dispersion, cell culture, and treatment

Single-cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as previously described in detail (26). 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 to 4 days the medium was changed and 72-hour incubations without or with test substances (OCT and PAS) were initiated. Both compounds were obtained from Novartis Pharma (Basel, Switzerland) and tested in quadruplicate (or triplicate, when total cell number was not sufficient) at the concentration of $10^{-8}$ M (10 nM). This concentration was based on previous studies testing the same molecules in the same adenoma cell type (23, 24). Moreover, the choice for a 72-hour incubation has been explained in detail in previous studies from our group (27). Cells were cultured at 37°C in a $CO_2$ incubator. 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 culture medium consisted of minimum essential medium supplemented with nonessential amino acids, sodium pyruvate (1 mmol/L), 10% fetal calf serum, penicillin ($1 \times 10^5$ U/L), fungizone (0.5 mg/L), L-glutamine (2 mmol/L), and sodium bicarbonate (2.2 g/L, pH 7.6). Media and supplements were obtained from Gibco Bio-cult Europe (Invitrogen, Breda, The Netherlands).

Finally, based on the variable effects of the two compounds observed in the different cell cultures, we aimed to identify three different adenoma subgroups: adenomas PAS+ (PAS was more effective than OCT), adenomas OCT+ (OCT showed higher efficacy than PAS), and OCT = PAS, when the effect of the two compounds in the single adenoma culture was equal. To define the possible superiority of a compound we used the following criteria: (1) the efficacy of one compound in reducing *in vitro* GH secretion was significantly higher compared with the other (based on statistical significance), or (2) only one of the two drugs was able to significantly decrease GH secretion compared with the control (based on statistical significance).

## Quantitative polymerase chain reaction

Quantitative polymerase chain reaction was performed according to a previously described method (23). Briefly, to perform SSTR membrane receptor mRNA evaluation, poly-$A^+$ mRNA was isolated from adenoma tissues using Dynabeads oligo$(dT)_{25}$ (Dynal, Oslo, Norway). Complementary DNA was synthesized using the poly-$A^+$ mRNA, which was eluted from the beads in 40 μL of $H_2O$ twice for 2 minutes at 65°C, using oligo$(dT)_{12–18}$ primer (Invitrogen). Samples were measured on an ABI Prism 7900 sequence detection system (PerkinElmer, Foster City, CA) for real-time amplifications, according to the manufacturer's protocol. The primer and probe sequences, the efficiencies, and the reaction conditions that were used for the detection of $sst_1$, $sst_2$, $sst_3$, $sst_5$, and human hypoxanthine phosphoribosyltransferase (hprt) have been previously described (23, 27). The detection of hprt served as control (housekeeping gene) and was used to normalize membrane receptor mRNA expression.

## Immunohistochemistry

Formalin-fixed paraffin-embedded tumor specimens were cut into sequential 4-μm-thick sections and deparaffinized and stained using a fully automated Ventana BenchMark ULTRA Stainer (Ventana Medical Systems, Tucson, AZ) according to the manufacturer's instructions at the pathology department. Binding of peroxidase-coupled antibodies was detected using 3,3′-diaminobenzidine as a substrate and the slides were counterstained with hematoxylin.

The rabbit monoclonal anti-$sst_2$ antibody (BioTrend, Köln, Germany) was used at a dilution of 1:25, whereas the rabbit monoclonal anti-$sst_5$ antibody (28) was used at a dilution of 1:50.

The immunoreactivity score (IRS), which ranges between 0 (no staining) and 12 (maximum staining), is a semiquantitative scoring system, which allows evaluation of both the intensity of the staining and the percentage of positive cells in the adenoma tissue slides. As previously described (9), the IRS is calculated by the product of the percentage of positive cells (4, >80%; 3, 51% to 80%; 2, 10% to 50%; 1, <10%; 0, 0%) and the intensity of the staining (3, strong; 2, moderate; 1, mild; and 0, no staining), which results in IRS scores between 0 (no staining) and 12 (maximum staining).

## Statistical analysis

SPSS 21.0 for Windows (SPSS, Chicago, IL) was used for statistical analyses, whereas graphs and figures were drawn by use of GraphPad Prism software version 5.02 (GraphPad Software, San Diego, CA). Quantitative data are presented as mean ± standard deviation when data distribution was normal; otherwise, median with range (minimum to maximum) was

used. A Kolmogorov–Smirnov test was used to check the normality of distribution of the continuous variables. When possible, log transformation was used to normalize the distribution of continuous nonparametric variables (*e.g.*, SSTR mRNA expression).

Between-group comparisons were analyzed by the two-tailed *t* test (normal distribution of the data), the Mann–Whitney *U* test (nonparametric data), or the Kruskal–Wallis test (nonparametric data, when we compared more than two groups). Accordingly, correlation coefficients were calculated using linear regression analysis or Spearman rank order R. Differences were taken to be statistically significant at $P < 0.05$.

## Results

### Patients and tumor characteristics

General, clinical, and biochemical characteristics of patients and functional information on the adenomas included in this study are summarized in Table 1.

Twenty-four out of 33 patients (73%) underwent presurgical medical treatment. Specifically, 13 were treated with SSAs alone (OCT long-acting release or LAN Autogel), nine with the combination of SSAs and pegvisomant (Peg, a GH-receptor antagonist), one with SSA (OCT long-acting release) combined with cabergoline (dopamine agonist), and one with Peg alone. Latest biochemical values measured before surgery (median, minimum to maximum) were: GH, 7.7 μg/L (0.7 to 252); absolute IGF-I, 82.1 nmol/L (23.1 to 344); and IGF-I ULNR, 2.24 (0.4 to 8.19). For GH evaluation, we excluded those patients treated with Peg in combination with SSAs, because the commercial assay we used to measure GH levels cross-reacts with Peg. However, as expected, preoperative GH levels were directly correlated with both absolute and ULNR-normalized IGF-I levels ($r = 0.627$, $P = 0.002$ and $r = 0.443$, $P = 0.039$, respectively). Moreover, absolute IGF-I levels were significantly lower in pretreated patients compared with the naive ones ($P = 0.038$), and GH levels showed a same trend, despite not reaching statistical relevance ($P = 0.064$). Preoperative serum PRL levels (available for 30 patients) were elevated in 23% (upper limit of normality 0.36 U/L), with a median value of 0.23 U/L (0.01 to 2.0). Serum PRL levels were above normal range in five of 11 (45%) patients whose tumors showed GH and PRL cosecretion *in vitro*, and in two of 19 (11%) patients harboring "pure" GH-secreting adenomas (only GH secretion detectable in cultured adenoma cells).

Immunohistochemical evaluation of pituitary samples showed the presence of a pituitary adenoma positive for GH staining in 30 out of 32 samples analyzed. Indeed, as above mentioned, the pathology report was not available for one patient and not conclusive in two patients. The concomitant presence of PRL immunoreactivity was found in 10 out of 30 adenomas (33%), ranging from sporadic PRL-positive cells to diffuse PRL positivity.

**Table 1. General, Clinical, and Biochemical Characteristics of Patients and Functional Information of the Adenomas Included in This Study**

| Data | Number, n (%)[a] |
|---|---|
| Patients | 33 |
| Age, y, median (range) | 42 (16–65) |
| Sex | M, 19 (58) |
| Tumor size | Macro, 24/32[a] (75) |
| Pre-NCH medical treatment | 24/33 (73) |
| SSAs | 13/24 (54) |
| SSAs + Peg | 9/24 (38) |
| SSAs + Cab | 1/24 (4) |
| Peg | 1/24 (4) |
| Pre-NCH biochemical levels | |
| GH, median (range) | 7.7 μg/L (0.7–252) |
| IGF-I, absolute, median (range) | 82.1 nmol/L (23.1–344) |
| IGF-I, ULNR, median (range) | 2.24 ULNR (0.4–8.19) |
| PRL, median (range) | 0.23 U/L (0.01–2.0) |
| Tumor characteristics | |
| GH secretion | 33/33 (100) |
| PRL secretion | 11/33 (33) |

Abbreviations: Cab, cabergoline; Macro, macroadenoma; NCH, neurosurgery (namely, transsphenoidal adenomectomy).

[a]Not all information is available for all patients.

### SSTR expression

SSTR mRNA expression was evaluated in all (n = 33) adenoma samples. In line with previous finding from the literature (29, 30), $sst_5$ and $sst_2$ were the most predominantly expressed SSTRs (relative expression, normalized to hprt), with median (minimum to maximum) values of 0.35 (0.0 to 1.08) and 0.20 (0.03 to 1.38), respectively. Median $sst_1$ expression was 0.01 (0.0 to 1.18), and $sst_3$ mRNA was expressed at a very low level (median, 0.007; 0.0 to 0.12). SSTR mRNA expression (for all ssts evaluated) did not significantly differ when comparing samples from SSA-pretreated and nonpretreated patients ($sst_1$, $P = 0.114$; $sst_2$, $P = 0.155$; $sst_3$ $P = 0.983$; and $sst_5$ $P = 0.603$).

As mentioned above, $sst_2$ and $sst_5$ expression was evaluated at protein level, as well. Median $sst_2$ IRS was 6 (1 to 12), whereas median $sst_5$ IRS was 12 (0 to 12). In line with previous data from the literature (8, 10), $sst_2$ IRS was significantly lower in adenoma samples from patients who underwent SSA presurgical medical therapy compared with treatment-naive patients ($P = 0.001$), whereas no statistically relevant differences were observed for $sst_5$ protein expression between the two groups. However, $sst_2$ and $sst_5$ IRS were directly correlated ($r = 0.412$, $P = 0.045$).

### Effects of OCT and PAS on *in vitro* GH and PRL secretion

Overall, *in vitro* efficacy of OCT and PAS ($10^{-8}$ M) in reducing GH levels (percentage vs control) after 72 hours incubation was superimposable. OCT treatment induced a

mean GH decrease of −36.8% ± 16.2%, whereas PAS reduced GH levels by −37.1% ± 15.7% [Fig. 1(a)]. The OCT effect ranged from −7% to −74%, whereas the PAS effect ranged from −7% to −81%. Moreover, the percentage GH decrease induced by OCT and PAS treatment was strongly and directly correlated when performing a pairwise comparison of the different 33 adenoma cell cultures ($r = 0.829$, $P < 0.0001$).

Based on the criteria described in *Patients and Methods*, we were able to identify six (18%) adenoma cultures where PAS was more potent than OCT in reducing GH secretion (PAS+ group), and five (15%) adenomas in which OCT was more effective than PAS (OCT+). In the remaining 22 primary cultures (67%), the efficacy of the two compounds did not significantly differ (OCT = PAS group) [Fig. 1(b)]. Specifically, mean GH reduction in the PAS+ group was −33.7% ± 7.8% for PAS and −19.8% ± 7.6% for OCT ($P = 0.0006$), whereas in the OCT+ group OCT treatment resulted in a mean GH lowering of −52.0% ± 13.4% and PAS in −39.6% ± 13.3% ($P = 0.0002$). Mean GH reduction in the OCT = PAS group was −37.9% ± 14.6% and −37.5% ± 18.0% for OCT and PAS, respectively [Fig. 1(c)].

As for the inhibition of PRL secretion (n = 11), PAS was significantly more effective compared with OCT (−52.4% ± 16.1% vs −34.3% ± 22.2%, $P = 0.003$) (Fig. 2). Of note, within the same group of 11 GH and PRL cosecreting adenomas, the efficacy of PAS and OCT in the reduction of GH secretion was not significantly different (−41.9% ± 14.6% vs −40.2% ± 13.5%) (Fig. 2).

## Correlation between adenoma SSTR expression and *in vitro* hormone secretion

To compute linear regression analysis, we performed log transformation of SSTR mRNA expression.

As expected, $sst_2$ (log) mRNA expression was significantly positively correlated with the ability of OCT to reduce *in vitro* GH secretion ($r^2 = 0.22$, $P = 0.005$), whereas only a slight trend for linear correlation was observed for PAS ($r^2 = 0.09$, $P = 0.086$) [Fig. 3(a) and 3(b)]. On the contrary, $sst_5$ (log) mRNA expression was not significantly correlated with the effect of either OCT or PAS on GH secretion [Fig. 3(c) and 3(d)]. The $sst_2/sst_5$ ratio (log) was positively correlated with the effect of OCT ($r^2 = 0.16$, $P = 0.031$), whereas no correlation was observed for PAS ($P = 0.38$).

Moreover, we observed that $sst_2$ mRNA levels were significantly higher in those adenoma cultures in which we found a ≥50% GH inhibition after both OCT or PAS treatment ($P = 0.032$ and $P = 0.004$, respectively).

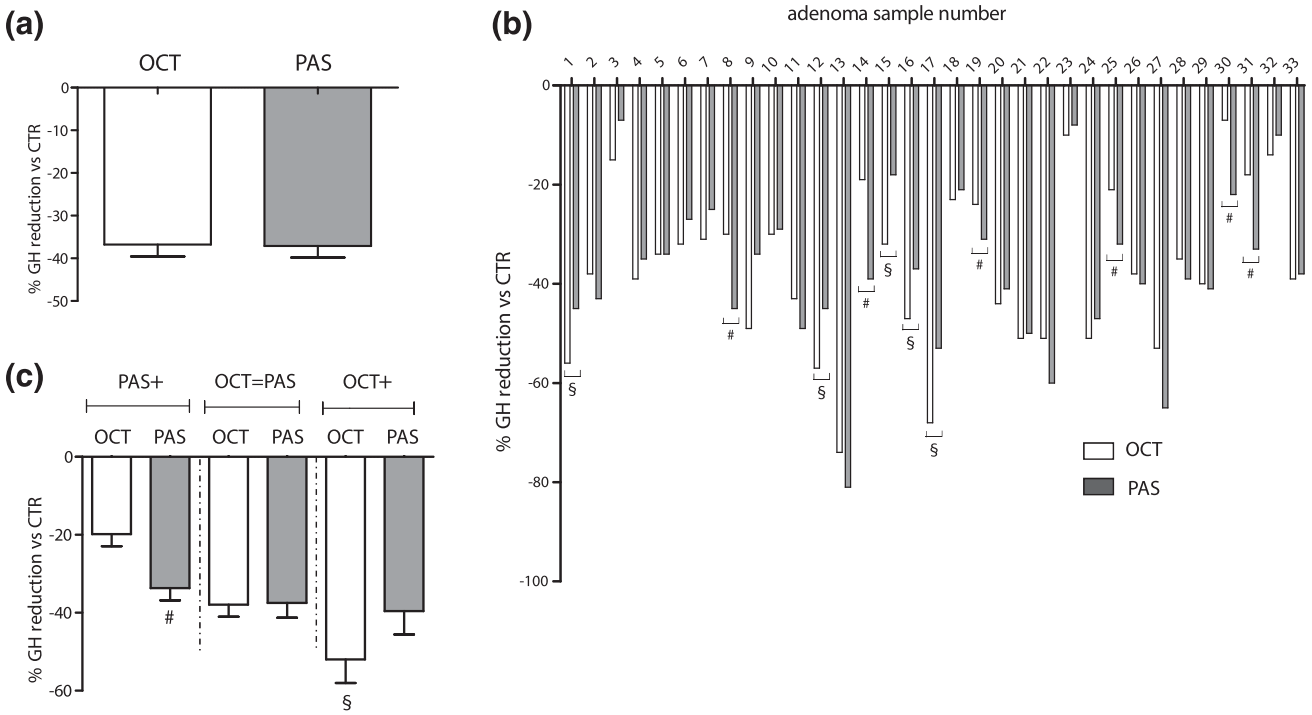


**Figure 1.** *In vitro* effect of OCT (10 nM) and PAS (10 nM) on GH secretion evaluated in 33 GH-secreting adenoma primary cultures. (a) Overall efficacy of the two drugs [expressed as mean percentage reduction vs control ± standard deviation (SD)] was superimposable after 72 hours incubation (OCT, −36.8% ± 16.2%; PAS, 37.1% ± 15.7%). (b) Detailed antisecretory effect of OCT and PAS in the different cell cultures. (c) Mean antisecretory effect (± SD) of OCT and PAS in the three adenoma subgroups is depicted. The subgroups have been defined based on the variable effect of the two compounds observed in the different cell cultures: adenomas PAS+ (PAS more effective than OCT), adenomas OCT+ (OCT has higher efficacy than PAS), and OCT = PAS, the effect of the two compounds is comparable. For more details see *Patients and Methods*. §, OCT more potent than PAS ($P < 0.05$); #, PAS more potent than OCT ($P < 0.05$); CTR, control.

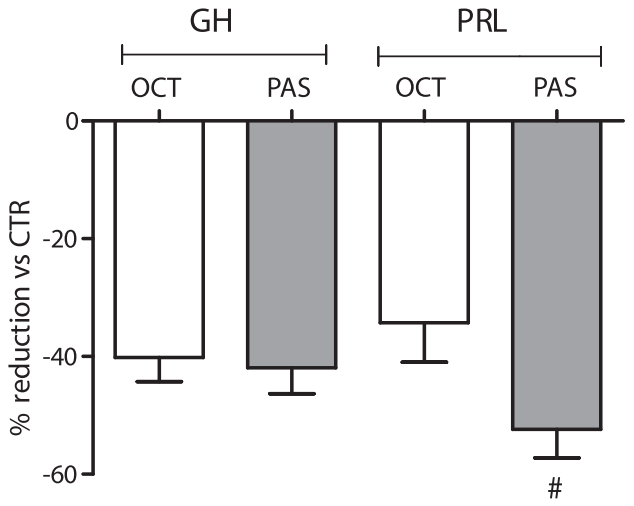


**Figure 2.** *In vitro* effect of OCT and PAS on GH and PRL secretion in the 11 cosecreting adenoma cultures is depicted (mean ± standard deviation percentage reduction vs control). Both drugs were tested at the concentration of 10 nM. #, PAS is significantly more potent than OCT in inhibiting PRL secretion ($P$ = 0.003). The effect of the two compounds does not differ significantly in reducing GH secretion. CTR, control.

Interestingly, this was not the case for $sst_5$ mRNA levels ($P$ = 0.853 and $P$ = 0.679, respectively). When using linear regression analysis, no correlation was found between $sst_1$ or $sst_3$ mRNA expression and GH reduction after OCT or PAS treatment (data not shown).

Of note, the inhibitory effect of PAS on GH *in vitro* secretion was significantly correlated with the rough sum (log) of all SSTRs, evaluated at mRNA level ($r^2$ = 0.14, $P$ = 0.04). Moreover, a same trend of correlation, although not reaching statistical significance, was found between the OCT effect and the sum of all SSTRs (log) ($r^2$ = 0.12, $P$ = 0.067).

As for the inhibition of PRL secretion, we found that none of the SSTRs evaluated (at mRNA level) correlated with the antisecretory effect of both OCT and, more surprisingly, PAS. However, we observed that $sst_1$ mRNA expression was significantly higher in the adenoma cultures secreting both GH and PRL, compared with the "pure" GH-secreting cells ($P$ = 0.026).

Finally, we correlated $sst_2$ and $sst_5$ protein expression (evaluated as IRS) with the *in vitro* GH secretion responsiveness to OCT and PAS treatment. We found that both OCT and, surprisingly, PAS efficacy was inversely correlated with $sst_5$ IRS ($r$ = −0.583, $P$ = 0.003 and $r$ = −0.559, $P$ = 0.004, respectively). Alternatively, no significant correlations were found between $sst_2$ IRS and OCT or PAS *in vitro* efficacy ($r$ = 0.169, $P$ = 0.431 and $r$ = 0.200; $P$ = 0.348, respectively).

## SSTR expression in the adenoma subgroups showing different response to OCT and PAS

As described in *Patients and Methods*, based on the variable effect of the two compounds observed in the different cell cultures, we were able to identify three different adenoma subgroups: adenomas PAS+ (n = 6), OCT+ (n = 5), and OCT = PAS (n = 22). We observed that $sst_2$ mRNA expression was relatively low in the

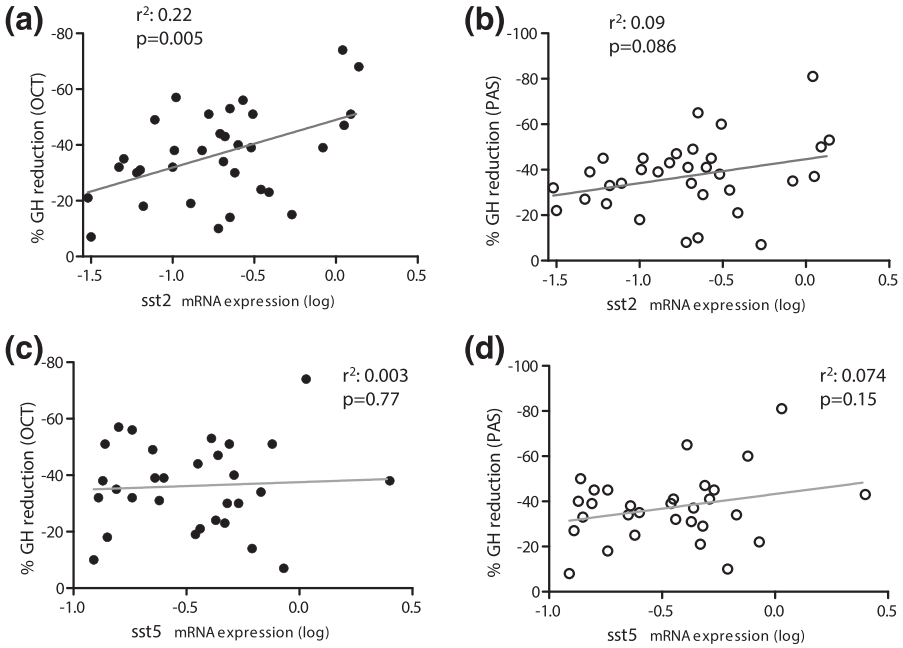


**Figure 3.** Linear regression analysis between $sst_2$ and $sst_5$ mRNA levels and the effect of OCT and PAS in inhibiting GH secretion. Both drugs were tested at the concentration of 10 nM. Because $sst_2$ and $sst_5$ mRNA values were variables not normally distributed, we performed log transformation to compute linear regression analysis. Linear correlation between $sst_2$ (log) and OCT and PAS percentage GH reduction (vs control) is depicted in (a) and (b), whereas $sst_5$ (log) correlation is presented in (c) and (d). Linear regression $r^2$ and related $P$ values are reported in each panel.

PAS+ group (median value, 0.06/hprt), higher in the OCT = PAS group (median, 0.21/hprt), and highest in the OCT+ group (median, 0.27/hprt), although these differences showed only a trend for statistical significance (Kruskal–Wallis test, $P = 0.059$) [Fig. 4(a)]. On the contrary, $sst_5$ mRNA expression was higher in PAS+ adenomas (median value 0.40/hprt), while showed the lowest levels in the OCT+ group (median 0.18/hprt). However, this difference was not statistically significant ($P = 0.274$) [Fig. 4(b)]. Finally, $sst_2/sst_5$ mRNA ratio was lower in the PAS+ group (median ratio, 0.24) and increased significantly in the other two groups (median, 0.49 and 1.1, respectively; $P = 0.037$) [Fig. 4(c)].

We also compared the SSTR mRNA expression between the PAS+ group and all the other samples (OCT = PAS plus OCT+ group). We observed that the PAS+ group had significantly lower $sst_2$ levels compared with the OCT = PAS plus OCT+ group (Mann–Whitney *U* test, $P = 0.024$) and lower $sst_2/sst_5$ ratio ($P = 0.041$) [Fig. 4(d) and 4(f)]. The $sst_5$ mRNA expression showed only a trend, although not statistically significant, for higher levels in the PAS+ group (median expression, normalized to hprt, 0.40 vs 0.24) [Fig. 4(e)].

Of note, no statistically relevant differences were observed for $sst_2$ and $sst_5$ IRS in the three adenoma subgroups (Kruskal–Wallis test, $P = 0.610$ and $P = 0.082$, respectively). In this context, we emphasize that, based on the subgroup stratification and the availability of immunohistochemical data for $sst_2$ and $sst_5$ protein staining, only two patients were included in the OCT+ group, thus limiting the power of the related statistical analyses.

## Discussion

In this study we compared the direct antisecretory effect of OCT and PAS in a large number of primary cultures of GH (PRL)-secreting adenomas with the expression of SSTRs, evaluated at both the mRNA and protein levels. Moreover, due to the large number of samples included in this study, based on strict statistical criteria, we were able to identify different adenoma subgroups, in which one of the two tested compounds showed higher efficacy compared with the other.

In our study we confirmed, *in vitro*, the results reported in the recent head-to-head superiority clinical study investigating the efficacy of OCT and PAS in the

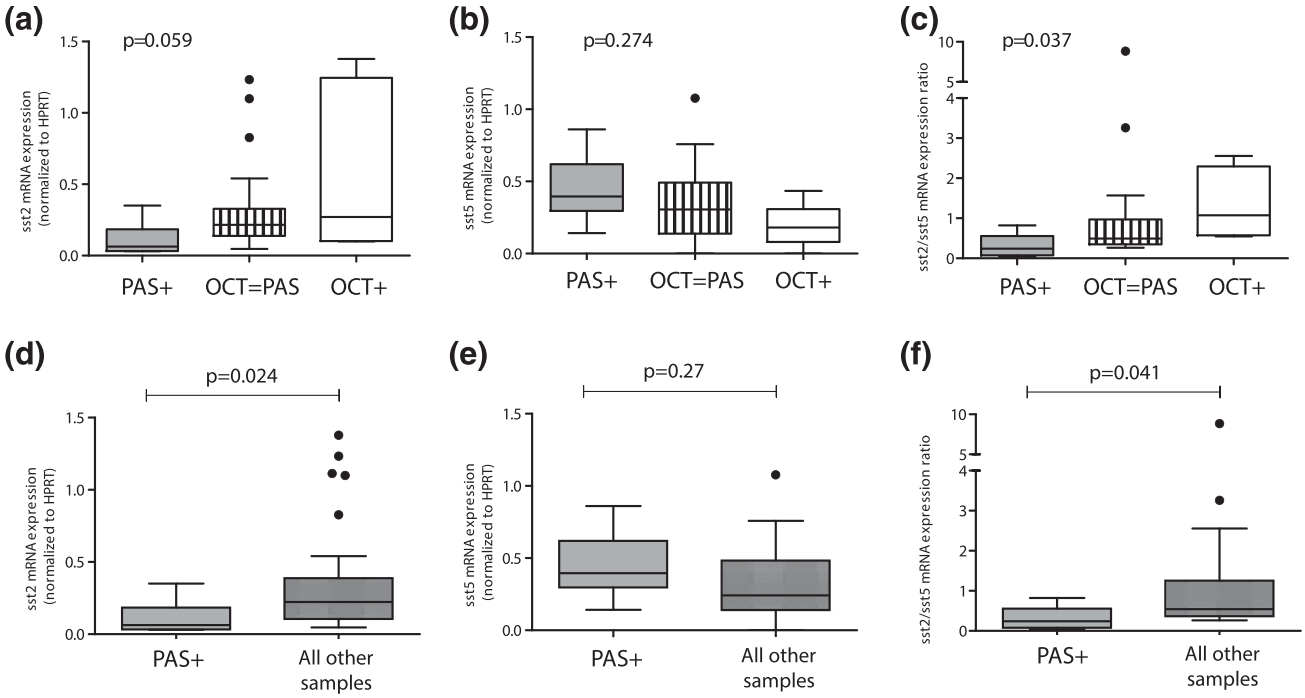


**Figure 4.** The $sst_2$ and $sst_5$ receptor expression in different adenoma subgroups identified based on OCT and PAS efficacy on GH secretion. Both drugs were tested at the concentration of 10 nM. (a) Levels of $sst_2$ mRNA in PAS+, OCT = PAS, and OCT+ groups. An opposite trend for $sst_5$ mRNA expression in the three groups is depicted in (b), whereas the values of the $sst_2/sst_5$ mRNA ratio are presented in (c). In (d–f), $sst_2$ and $sst_5$ mRNA expression (together with $sst_2/sst_5$ mRNA ratio) between the PAS+ group and all the other samples (namely, OCT = PAS plus the OCT+ group) are depicted, as well. (d) The PAS+ group shows significantly lower $sst_2$ levels and (f) lower $sst_2/sst_5$ ratio compared with the merged OCT = PAS and OCT+ groups. The lower and upper bars represent the 25th and 75th percentiles (interquartile range), respectively. The lines across the box represent median value. The lines above and below the box represent 75th percentile plus 1.5 times interquartile range and the 25th percentile minus 1.5 interquartile range, respectively. (a–c) Black dots represent all data above or below these values. Statistical significance was determined using the Kruskal–Wallis test when the receptor expression in the three groups was analyzed, and (d–f) by use of the Mann–Whitney *U* test when comparing the two different groups. Related *P* values are reported in each panel.

treatment of patients with acromegaly, where the effect of the two drugs in the reduction of GH levels was comparable (whereas *in vivo* PAS was more effective in reducing IGF-I levels) (17). Indeed, in line with this finding, we observed that the overall *in vitro* efficacy of the two drugs in reducing GH secretion was superimposable. Of note, this observation is supported by a recent study by Ibañez-Costa *et al.* (31).

Additionally, we confirmed the already well-established correlation between $sst_2$ mRNA expression and the *in vitro* antisecretory effect of OCT in primary cultures of GH-secreting adenomas.

Interestingly, the effect of PAS showed a slight trend for correlation with $sst_2$ mRNA expression (without reaching statistical significance), in line with a previous observation in a small series of adenomas (23). Moreover, $sst_2$ mRNA levels were significantly higher in the adenoma cultures in which we found a $\geq$50% GH inhibition after PAS treatment compared with those cell cultures showing a PAS-induced GH decrease of $<$50%. These findings are further supported by the strong and direct correlation we observed between the effect of PAS and OCT on GH secretion in the different adenoma cell cultures. Taken together, these observations suggest an important role of $sst_2$ in the PAS-induced antisecretory effect in GH-secreting adenomas as well. Despite that observations in *in vitro* studies carried out on transfected cell lines have demonstrated biased agonist properties for PAS in respect to $sst_2$ (*e.g.*, slightly less potent effect on cyclic adenosine monophosphate inhibition, no effect on intracellular $Ca^{2+}$ modulation) (19), we can speculate that other properties of the ligand, for example the reduced activation of receptor internalization, together with its faster recycling on the cell membrane (20), can counterbalance the absolute less powerful activation of the second messenger pathways, particularly during prolonged drug exposure (21).

Alternatively, in the adenoma samples included in the present study (from patients not preselected based on their responsiveness to first-generation SSAs), we did not observe any statistically relevant linear correlation between the *in vitro* effect of PAS on GH secretion and $sst_5$ mRNA expression. In this respect, we just observed that tumors with higher $sst_5$ mRNA expression or lower $sst_2/sst_5$ ratio seem to better respond to PAS compared with OCT. These latter observations are in line with the recent data from Iacovazzo *et al.* (32), reporting a correlation between PAS responsiveness (*in vivo*) and a higher adenoma $sst_5$ expression in a specific subgroup of patients (n = 11), resistant to first-generation SSAs.

In line with the significant correlation observed between PAS effect and the rough sum of the SSTRs evaluated at mRNA levels (all but $sst_4$), we can also hypothesize that, in GH-secreting adenomas, PAS acts as a peculiar SSTR pan-ligand, with a preferential action on $sst_2$, although able to activate a number of different SSTR subtypes (*e.g.*, $sst_5$). This speculation is supported by the analysis of the adenoma stratification we performed in the present study, based on the variable effect of the two compounds observed in the different cell cultures. Indeed, the absolute effect of OCT (percentage GH decrease vs control) significantly differs between PAS+, OCT = PAS, and OCT+ groups, whereas the effect of PAS is clearly less variable in the different groups [see Fig. 1(c)]. In this light, PAS comes out as a less potent but more versatile compound compared with OCT.

Moreover, based on the above described findings and the evaluation of SSTR mRNA expression in the different adenoma subgroups, PAS seems to be the drug of choice in those GH-secreting adenomas showing relatively low $sst_2$ mRNA and low $sst_2/sst_5$ ratio, whereas OCT should be preferred in the presence of high $sst_2$ mRNA levels.

Alternatively, we have to point out that the correlation between $sst_2$, $sst_5$ protein expression, and the *in vitro* antisecretory effect of the two drugs is difficult to interpret. The lack of correlation between $sst_2$ IRS and the *in vitro* efficacy of OCT can be explained by the pooled evaluation of both patients treated with SSAs (and Peg) before surgery and treatment-naive patients. Because it is known (8–10) (and confirmed in the present study) that $sst_2$ protein expression in adenoma samples from pretreated patients is significantly lower compared with the untreated ones, this could be an important bias affecting the correlation analysis. As for $sst_5$ protein expression, it is not affected by SSA presurgical medical treatment (8, 10). Although the inverse correlation found between OCT effect and $sst_5$ IRS is somewhat expected (11), the observation of the same correlation for PAS is somehow surprising.

A general issue, possibly related to the lack of correlation between mRNA and protein data, already reported by other authors (33), is the fact that these two different techniques are performed on different pieces of the same adenoma, which can show a heterogeneous SSTR expression in its different sections.

To summarize, we think that in the present experimental setting, the evaluation of SSTR mRNA expression by quantitative reverse transcription polymerase chain reaction, directly performed on the same dispersed adenoma cells afterward plated to evaluate the *in vitro* effect of the two tested compounds, can provide clearer indications about the role of the different receptors, compared with the immunohistochemical evaluation on paraffin-embedded tissues. Of course, we think this is not the case when analyzing the role of SSTR in driving the *in vivo* responsiveness to SSAs.

As for PRL cosecretion, present *in vitro* in 11 samples, we confirmed (23, 24) that PAS has a more effective antisecretory activity compared with OCT. A possible explanation for this finding is the higher $sst_1$ mRNA expression observed in the GH-PRL–secreting tumors, compared with the pure GH-secreting ones. Indeed, due to the peculiar compound binding affinities, $sst_1$ is a potential target for PAS but not for OCT treatment. Of note, $sst_1$ is also one of the mostly expressed SSTRs (together with $sst_5$) in prolactinomas (34). However, $sst_5$ mRNA expression was not statistically different between GH-PRL–secreting tumors and the pure GH-secreting ones (data not shown).

In our opinion, the main strength of our study is represented by the large number of primary cultures in which the antisecretory effect of OCT and PAS has been systematically compared head-to-head, together with the availability of enough tissue to directly perform the evaluation of SSTR mRNA expression on the same dispersed adenoma cells for all samples included in the study. Alternatively, the main limitation of our study resides in the lack of SSTR protein data from dispersed adenoma cells, although it was available from paraffin-embedded tissues. The availability of sufficient additional fresh adenoma tissue would have allowed us to perform additional experiments (*e.g.*, Western blot analysis) to investigate the protein expression of SSTRs in our cell cultures. Unfortunately, this was not possible in our samples.

In conclusion, our study shows that the *in vitro* effect of OCT and PAS in reducing GH secretion from cell cultures of GH-secreting adenomas is overall superimposable. Alternatively, PAS is more effective than OCT in reducing the concomitant PRL secretion, when present. Moreover, we have been able to identify different adenoma subgroups, in which the effect of one of the two compounds is significantly more potent that the other one. In this context, we found that adenomas with lower $sst_2$ mRNA expression and lower $sst_2/sst_5$ ratio are most likely to be better responders to PAS, in terms of *in vitro* GH secretion.

All of these findings need to be further explored and translated into a more clinical context, because they open an intriguing scenario for the future of medical treatment in acromegaly. The evaluation and the analysis of SSTR expression in tumor samples from an adequate cohort of PAS-treated patients could drive us in the next future to choose the best SSA for the adjuvant treatment of acromegaly, tailored to patient's tumor characteristics.

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