Octreotide and pasireotide (dis)similarly inhibit pituitary tumor cells in vitro

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Abstract

Somatostatin analogs (SSA) are the mainstay of pharmacological treatment for pituitary adenomas. However, some patients escape from therapy with octreotide, a somatostatin receptor 2 (sst2)-preferring SSA, and pasireotide, a novel multi-sst-preferring SSA, may help to overcome this problem. It has been proposed that correspondence between sst1-sst5 expression pattern and SSA-binding profile could predict patient’s response. To explore the cellular/molecular features associated with octreotide/pasireotide response, we performed a parallel comparison of their in vitro effects, evaluating sst1-sst5 expression, intracellular Ca2+ signaling ([Ca2+]i), hormone secretion and cell viability, in a series of 85 pituitary samples. Somatotropinomas expressed sst5>sst2, yet octreotide reduced [Ca2+]i more efficiently than pasireotide, while both SSA similarly decreased growth hormone release/expression and viability. Corticotropinomas predominantly expressed sst5, but displayed limited response to pasireotide, while octreotide reduced functional endpoints. Non-functioning adenomas preferentially expressed sst3 but, surprisingly, both SSA increased cell viability. Prolactinomas mainly expressed sst1 but were virtually unresponsive to SSA. Finally, both SSA decreased [Ca2+]i in normal pituitaries. In conclusion, both SSA act in vitro on pituitary adenomas exerting both...

Key Words
- pituitary
- pituitary adenoma
- somatostatin
- gene expression
- cell signaling
similar and distinct effects; however, no evident correspondence was found with the sst1-sst5 profile. Thus, it seems plausible that additional factors, besides the simple abundance of a given sst, critically influence the SSA response.

Introduction

Pituitary tumors are generally benign adenomas from a monoclonal/oligoclonal origin, which display heterogeneous clinical manifestations derived from oversecretion of a single hormone and/or size effects due to excess growth (Melmed 2011). Surgery is the first-line treatment for most pituitary tumors, except for prolactin (PRL)-secreting lactotrope tumors (prolactinomas), which often respond favorably to medical treatment with dopamine agonists. Synthetic somatostatin (SST) analogs (SSA) represent a valuable first-line medical treatment for various types of pituitary tumors, particularly in GH- and thyrotrophin (TSH)-secreting tumors (called somatotropinomas and thyrotropinomas, respectively), and, potentially also, in adrenocorticotropic hormone (ACTH)-secreting tumors (corticotropinomas), owing to their abundant expression of SST receptors (sst1, sst2, sst3, sst4 and sst5; codified by SSTR1, SSTR2, SSTR3, SSTR4 and SSTR5 genes). These receptors are commonly co-expressed, simultaneously, at different levels in normal pituitary cells, where they convey SST actions to regulate hormone release (Theodoropoulou & Stalla 2013). In pituitary tumors, previous studies (Taboada et al. 2007, Neto et al. 2009, Hofland et al. 2010a, Chalabi et al. 2014) have reported different expression patterns of sst1-sst5 depending on the type of tumor: high sst2-sst5 expression is typical in GH- and TSH-secreting adenomas; sst5 predominates in corticotropinomas; sst1-sst5 expression in prolactinomas; and sst3-sst2 expression in gonadotrope lineage-derived tumors, which frequently lack hormone oversecretion and are thus known as non-functioning pituitary adenomas (NFAs). The ability of ssts to activate various, often overlapping and cross-talking signaling pathways provides the basis for SSA inhibition of hormone secretion, cell proliferation and tumor growth. Indeed, widely used sst2-prefering SSA (octreotide, lanreotide) represent versatile therapeutic tools in acromegaly and thyrotropinomas (Theodoropoulou & Stalla 2013). Unfortunately, a relevant proportion of patients are (or become) partially or totally resistant to these drugs, while other pituitary tumors are largely unresponsive to SSA (Colao et al. 2011, Theodoropoulou & Stalla 2013), which likely relates to the specific presence, abundance, availability and/or signaling properties of sst1-sst5 expression in each particular tumor. To circumvent this problem, novel analogs with multireceptor-binding profiles have been developed, such as pasireotide. This SSA binds with high affinity not only to sst5, but also to sst2 and sst3 (and less potently to sst1), and is already being applied to treat pituitary tumors in clinical practice, as it controls a relevant proportion of corticotropinomas in contrast to octreotide (Boscaro et al. 2009). In addition, the superiority in clinical efficacy of pasireotide vs octreotide has been shown in treatment-naïve acromegaly patients (Colao et al. 2014) as well as in patients who were resistant to octreotide or lanreotide (Gadelha et al. 2014).

Despite recent advances in clinical SSA development, key aspects of the mechanisms mediating the effects of different SSA on pituitary tumors remain incompletely understood. Indeed, few studies have explored the actual differences/similarities of the direct actions of sst2-prefering and multi-sst SSA on the different pituitary tumor types, which may not solely depend on their distinct sst-binding profile. Besides, a number of different factors substantially influence the functional capacities of a given analog, from receptor internalization, recycling, degradation or interaction, to selectivity in signaling pathway activation, thereby defining precisely their actions, which would also depend on the target cell type (Schonbrunn 2008). Accordingly, we have herein implemented a systematic analysis of the direct in vitro actions of octreotide and pasireotide in a representative series of the main classes of pituitary tumors, as well as in non-tumoral human pituitary. Unlike previous reports comparing the in vitro actions of these SSA, which mostly studied separately a single type of pituitary adenoma (Stalla et al. 1994, Hofland et al. 2004, Hofland et al. 2005, van der Hoek et al. 2005, Zatelli et al. 2007, van der Pas et al. 2013), here we deploy an integrative methodology to evaluate, in parallel, the in vitro response of human primary pituitary tumor cell cultures to both SSA by assessing several functional parameters, which include key aspects in pituitary tumor pathology. Thus, ssts expression analysis using quantitative real-time PCR (qPCR)
was performed on the tissue samples, wherein we measured SSA-induced kinetics of free cytosolic calcium concentration ([Ca^{2+}]_i), a key signaling pathway that is effected upon ligand binding to sst receptors to control hormone exocytosis and cell proliferation/apoptosis, as well as the two main outputs in endocrine tumors, hormone release and cell viability, as suitable markers for the secretory and growth response of the tumors.

**Materials and methods**

**Reagents**

Unless otherwise indicated, reagents and products were purchased from Sigma-Aldrich. Octreotide was obtained from GP-Pharm (Barcelona, Spain) and pasireotide was provided by Novartis. Both compounds were administered at 100 nM as reported previously in a comparative study (van Hoek et al. 2009), in which other concentrations were unable to exert any effect in vitro.

**Patients, tissue collection and pituitary cell culture**

All experimental protocols were approved by the University of Cordoba/Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC) licensing committee. This study has been performed in accordance with the Declaration of Helsinki. Pituitary specimens were obtained by transsphenoidal surgery from 79 pituitary adenomas (32 somatotropinomas, 15 corticotropinomas, 28 NFPAs and 4 prolactinomas), after informed consent was provided by each patient and with the approval of the University of Córdoba/IMIBIC and Hospital Ethics Committees. Additionally, six normal pituitaries resected during surgical removal of a pituitary adenoma were also used. In all cases, tissue phenotype confirmation was supported by three separate methods as described previously (Luque et al. 2013): detailed histological examination by an anatomopathologist, testing the hormonal phenotype using single-cell secretion on cells seeded onto PVDF membranes to evaluate the secretion of all pituitary hormones using specific antibodies, as previously reported (Vazquez-Martinez et al. 2008, Luque et al. 2013, Diaz-Rodríguez et al. 2014); and molecular screening by qPCR, as shown in Fig. 1. Normal pituitary tissues were collected during the resection of microadenomas (size<1 cm) derived from five corticotropinomas and one somatotropinoma, and were classified as normal tissue by these three methods, and confirmed by the presence of the adenoma in post-surgical imaging studies and biochemical analyses. Available demographic, clinical data and pretreatment therapies are summarized in Table 1. Particularly, each pituitary piece was placed after surgery in sterile cold S-MEM medium (Gibco) complemented with 0.1% BSA, 0.01% l-glutamine, 1% antibiotic-antimycotic solution and 2.5% HEPES and dispersed into single cells within the following 1–3 h as reported previously (Martínez-Fuentes et al. 2006, Luque et al. 2013, Ibáñez-Costa et al. 2015).

**RNA isolation, RT and qPCR**

Total RNA extraction, quantification, RT, qPCR procedure and primer sequences used to measure mRNA expression of the genes included in this study (SST1-SSTR5, GH, PRL, pro-opiomelanocortin (POMC), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyrotrophin (TSH) and α-subunit of glycoproteins (CGA)) and ACTB have been performed previously (Taboada et al. 2007, Neto et al. 2009, Luque et al. 2013, Ibáñez-Costa et al. 2015).
Measurement of free cytosolic calcium concentration ([Ca\(^{2+}\)]\(_i\))

Cells were plated on coverslips at a density of 50,000 cells per well and changes in [Ca\(^{2+}\)]\(_i\) after treatment with octreotide and pasireotide were tracked in single cells using fura-2/AM (Molecular Probes) as described previously (Martínez-Fuentes et al. 2006, Durán-Prado et al. 2009, Luque et al. 2013, Ibáñez-Costa et al. 2015).

Evaluation of hormone release

To examine the effects of SSA on pituitary hormone release, 100,000–150,000 cells per well were used. First, cells were plated in supplemented DMEM + 10% fetal bovine serum (FBS) for 24–36 h, and then were washed and serum starved for 1 h and incubated with octreotide or pasireotide (somatotropinomas: 4 and 24 h; corticotropinomas: 24 h incubation) in absence of FBS. After treatment, media were collected and stored at −20°C until hormone measurement using commercial ELISAs (GH and ACTH (EIA-3552 and EIA-3647, respectively; DRG, Marburg, Germany)). In somatotropinomas, whenever possible, cells were recovered for RNA analysis (see the ‘RNA isolation, RT and qPCR’ section above). All the information regarding each assay can be accessed at the company website.

Analysis of cell viability

As described previously (Durán-Prado et al. 2009, Luque et al. 2013, 2015, Ibáñez-Costa et al. 2015), cell viability was estimated using alamar-blue reagent (10,000 cells per well plate; Biosource International, Camarillo, CA, USA) in response to octreotide/pasireotide.

Statistical analysis

Statistical analyses were performed by unpaired parametric t-test and non-parametric Mann–Whitney U test according to normality, assessed by Kolmogorov–Smirnov test. We compared the effect of octreotide or pasireotide vs vehicle-treated control (set at 100%). Data were expressed as mean ± S.E.M. A P value of <0.05 was considered to be significant. All statistical differences were assessed using GraphPad Prism 6.

Results

The main aim of this study was to perform a systematic, comparative analysis of the effects of two SSA with distinct sst1-sst5 binding profile, octreotide and pasireotide on basic functional parameters in the main types of pituitary adenomas after determining the particular expression profile of sst.
Effects of octreotide and pasireotide on somatotropinomas

Regarding their hormonal phenotype, somatotropinomas (n=32) expressed GH exclusively and not PRL (only present in 20% of adenomas; Fig. 1A). They displayed, on average, high expression levels of sst5 and sst2, followed by sst3 and sst1 (Fig. 2A), which suggests a putative effectiveness of SSA, especially pasireotide, on these tumors. Calcium is a pivotal second messenger involved in the regulation of pituitary cell pathophysiology, which is required to trigger and sustain hormone release and other cell processes (e.g., apoptosis), and thus, it is widely used as an indicator to assess pituitary cell response to pharmacological treatment (Martinez-Fuentes et al. 2006, Ibáñez-Costa et al. 2015). Octreotide and pasireotide similarly decreased, on average, [Ca^{2+}]i (Fig. 2B). However, adenomas appeared to be more responsive to octreotide than to pasireotide, in terms of the overall proportion of tumors showing a [Ca^{2+}]i response to each drug, and in the percentage of responsive cells within each tumor (Fig. 2B). Similarly, both SSA decreased GH release in 2/2 cultures to a similar extent after 4 h treatment (Fig. 2C, left panel), to octreotide in 7/7, and to pasireotide in 5/5 cell cultures after 24 h (Fig. 2C, right panel), compared with vehicle-treated cells. Likewise, both SSA decreased GH, but not PRL mRNA levels after 24 h incubation without altering POU1F1 levels, the transcription factor responsible for GH and PRL expression (Fig. 2E, left panel). In contrast, while pasireotide treatment drastically reduced sst2 levels and induced an apparent, non-significant increase on sst5 levels (P=0.07), octreotide did not significantly alter the expression levels of any ssts (Fig. 2E, right panel). In addition, octreotide slightly, but significantly, decreased cell viability in 13/13 cell cultures after 24 h, in 9/9 after 48 h and 8/9 after 72 h, whereas pasireotide decreased cell viability in 13/13 after 24 h, in 9/9 after 48 h and 8/9 after 72 h (Fig. 2D).

Effects of octreotide and pasireotide on corticotropinomas

Corticotropinomas (n=15) expressed, on average, high levels of sst5, followed by sst2, which would enable a positive response to SSA, especially pasireotide (Fig. 3A). In terms of hormone expression, corticotropinomas expressed almost exclusively high POMC levels, with only some tumors moderately expressing PRL (Fig. 1B). Both SSA were able to decrease [Ca^{2+}]i, kinetics in vitro in roughly half of the corticotropinomas (4/10 and 5/9, respectively); yet, unexpectedly, octreotide appeared to be more efficient than pasireotide, as it exerted a greater degree of inhibition in a higher percentage of cells (Fig. 3B), and in a shorter period. Accordingly, 24 h incubation with octreotide inhibited ACTH release in two of the three tumors studied, while pasireotide only induced a non-significant reduction in two of the four tumors (Fig. 3C). Similarly, octreotide induced a moderate, albeit significant reduction in cell viability in two of the three tumors (P<0.05), whereas pasireotide did not evoke any reduction in cell viability in the two tumors studied (Fig. 3D).
Effects of octreotide and pasireotide on NFPAs

The NFPAs analyzed in this study were characterized by high sst3 expression, followed by sst2, sst5, sst1 and sst4 (n=28; Fig. 4A), which might suggest that pasireotide could be more effective than octreotide. As expected, CGA (α-subunit of the glycoproteins), FSH and LH were highly expressed (Fig. 1C). Cultured cells derived from NFPAs were rarely and poorly responsive to both SSA, nevertheless, octreotide appeared more effective than pasireotide, although it only inhibited \([\text{Ca}^{2+}]_i\) kinetics moderately, in a discrete subset of cells (Fig. 4B). Conversely, both compounds exerted clear and divergent responses in terms of cell viability, which were consecutively classified as ‘inhibitory responders’ or ‘stimulatory responders’. Specifically, octreotide tended to moderately (non-significantly) decrease cell viability in 5/16 cell cultures after 24h, in 2/8 after 48h and in 2/7 after 72h; while pasireotide only appeared to exert comparable, also non-significant actions on cell viability in 4/15 cell cultures after 24h, in 1/7 after 48h, and in 1/4 after 72h (Fig. 4C). Conversely, octreotide consistently induced moderate, albeit significant increases in cell viability compared with vehicle-treated cells in 11/16 cell cultures after 24h, in 5/8 after 48h and in 2/6 after 72h. Likewise, pasireotide significantly augmented cell viability in 11/15 NFPAs after 24h, in 6/7 after 48h and in 3/4 after 72h (Fig. 4D).

Effects of octreotide and pasireotide on prolactinomas

Prolactinomas exhibited high sst1 expression, with lower levels of other ssts (n=4; Fig. 5A), and very high expression of PRL, exclusively (Fig. 1D). Cells derived from these prolactinomas, which were resistant to in vivo treatment with cabergoline, displayed poor responses to SSA in vitro. Specifically, octreotide decreased \([\text{Ca}^{2+}]_i\), in 2/4 cultures, but only in a very low percentage of cells (7.0%), whereas it moderately increased \([\text{Ca}^{2+}]_i\) in 1/4 cell cultures, also in a low percentage of cells (Fig. 5B). In contrast, pasireotide did not evoke any appreciable \([\text{Ca}^{2+}]_i\) response (Fig. 5B). Moreover, both SSA decreased cell viability only in 1/3 tumors after 48–72h (Fig. 5C).

Effects of octreotide and pasireotide on normal pituitary

Normal pituitaries featured high sst5 expression, with lower sst2 and very low sst3 and sst1 levels (n=6; Fig. 6A). In terms of hormonal phenotype, as expected, GH and
that, in general, they compare well with those described previously for each of the tumor types examined (Taboada et al. 2007, Neto et al. 2009, Hofland et al. 2010a), supporting the notion that the samples investigated provide a representative picture of the pituitary tumor types under study. Although mRNA levels may not always reflect the precise functional protein levels in a cell (and in this case protein determination was a technically challenging assay that we could not perform given the limited tissue availability) the use of qPCR to assess mRNA levels has been shown as a good alternative approach, as a proxy for protein presence, according to recent studies (Vogel & Marcotte 2012). Nevertheless, it is important to note that the precise sst1-sst5 profile in each tumor did not always match, necessarily, that observed, on average, for its corresponding tumor type. It is widely and reasonably assumed that the actions of a specific SSA in a given pituitary tumor would result from the functional correspondence between the sst1-sst5-binding profile of that SSA and the pattern of sst1-sst5 expression in the tumor. Accordingly, it has been suggested that, in the future, recommendations to select a specific SSA could be done on the basis of sst expression pattern, for example, octreotide/lanreotide would be recommendable when sst2 is highly and predominantly expressed, whereas high expression of sst5, and sst2, sst3 or sst1 in pituitary adenomas would predict pasireotide responsiveness (Chalabi et al. 2014). However, this logical contention, which likely applies in general terms when large numbers of tumors or patients are considered on average, might not be similarly evident on an individual tumor basis, and, anyhow, such a theoretical assumption has not been unequivocally demonstrated through experimental testing so far. In fact, previous studies have indicated that sst2 presence in somatotropinomas positively correlated with in vivo SSA response (Taboada et al. 2008, Gatto et al. 2013), and a recent study revealed that octreotide-responsive patients were characterized by high sst2 and low sst5 presence, while octreotide-resistant patients showed high sst5 expression (Gonzalez et al. 2014). However, in this study, although somatotropinomas were characterized by sst5>sst2 expression, as described previously (Taboada et al. 2007, 2008, Hofland et al. 2010a), we observed that both SSA induced in vitro comparable inhibition of both GH release and cell viability. Moreover, it was noteworthy that pasireotide evoked less responses from cells than octreotide in terms of \([\text{Ca}^{2+}]\), kinetics, which would argue against the theoretical superiority of pasireotide predicted by the higher sst5 levels observed in these GH tumors. The reason for these latter differences is still unknown,

**Discussion**

Quantitative assessment of hormonal and sst1-sst5 expression profiles in the present tumor series revealed

Figure 5

PRL-secreting adenomas. (A) sst mRNA expression profile adjusted by ACTB \((n=4)\). (B) Summarized table of \([\text{Ca}^{2+}]\), kinetics assay. (C) Cell viability in response to octreotide and pasireotide \((n=3/3)\) at 24/48/72 h, respectively. For further details see Fig. 2 legend.

PRL were highly expressed, followed by POMC, CGA and LH/FSH, with faint TSH expression (Fig. 1E). Octreotide appeared less effective than pasireotide in decreasing \([\text{Ca}^{2+}]\). However, in responsive cultures, octreotide affected more cells and caused reductions of comparable amplitude to pasireotide (Fig. 6B). Besides, pasireotide, but not octreotide, significantly decreased cell viability after 24, 48 and 72 h (Fig. 6C).

Figure 6

Normal pituitary. (A) sst mRNA expression profile adjusted by ACTB \((n=6)\). (B) Summarized table of \([\text{Ca}^{2+}]\), kinetics assay. (C) Cell viability in response to octreotide and pasireotide \((n=2/1/1)\) at 24/48/72 h, respectively. For further details see Fig. 2 legend.
but could relate to the existence of distinct, tumor-specific sst1-sst5 distribution patterns, receptor-agonist and receptor–receptor interactions and dynamics, resulting in activation of different signaling pathways. In any case, our data suggest that the mere abundance of a given sst would not provide sufficient support to explain the level of response of a somatotropinoma to a SSA with strong affinity for that specific receptor, and, thus, additional mechanisms are required to explain the precise actions of each SSA in a target cell.

Nevertheless, our in vitro results are in line with the recent comparative in vivo analysis of octreotide and pasireotide treatment (Colao et al. 2014), where both compounds suppressed similarly GH levels, in that case, being pasireotide more efficient than octreotide, since more patients were biochemically controlled after 12 month treatment (Colao et al. 2014, Gadelha et al. 2014, Samson 2015). Interestingly, in our study, differences between the actions of both SSA were found in the control of gene expression, since both analogs similarly decreased GH mRNA, but, while octreotide did not appear to alter sst expression, pasireotide decreased sst2 levels and moderately augmented sst5 expression, a receptor-specific positive feedback effect that could potentially contribute to enhance in vivo responsiveness to this SSA (Colao et al. 2014). Thus, our results illustrate a substantial similitude between octreotide and pasireotide actions on GH secretion and cell survival in somatotropinomas in vitro, with some differences in regulation of Ca^{2+} signaling and sst expression. This suggests that both analogs may exert comparable effects at pituitary level in somatotropinomas, thus raising the possibility that some of the differences observed in vivo may relate to extrapituitary effects, which could help to explain the stronger inhibitory effect of pasireotide vs octreotide on insulin-like growth factor (IGF1) in patients with acromegaly in vivo (Colao et al. 2014).

Analysis of corticotropinomas showed the typical sst5-predominant profile, yet, results on [Ca^{2+}]_i kinetics, ACTH release and cell survival demonstrated that these tumors are significantly responsive, in vitro, to octreotide, similar to that previously reported on primary cultures (Hofland et al. 2005, 2010b) and in AtT-20 cells (Hofland et al. 2005, 2010b, van der Hoek et al. 2005). Our data also support the notion that octreotide exerts divergent actions in vivo and in vitro on corticotropinomas. Indeed, it is well known that glucocorticoids downregulate sst2, affecting octreotide response (van der Hoek et al. 2005, van der Pas et al. 2013), which suggests that octreotide therapy in untreated Cushing’s disease patients presenting with high cortisol levels would be ineffective as previously reported in two small series of untreated patients (Ambrosi et al. 1990, Stalla et al. 1994). Additionally, a recent study, using corticotropinoma cell cultures demonstrated that in cortisol-normalized patients, sst2 expression is increased at mRNA (van der Pas et al. 2013), which led to suggest that a pharmacological treatment to decrease cortisol levels (e.g., ketoconazole), followed by octreotide may be effective. The reason for the clear responses observed in this study to octreotide may relate to a recovery of sst2-related responsiveness, but the role of other ssts, particularly sst5, should not be discarded. In contrast, pasireotide did not induce significant responses in terms of ACTH release or cell viability in the corticotropinomas studied, where only a faint effect on [Ca^{2+}], kinetics was observed. These results are not in line with previous in vitro studies (Hofland et al. 2005, 2010b, van der Hoek et al. 2005, van der Pas et al. 2013), wherein pasireotide reduced ACTH release. However, the lack of effect of pasireotide on these corticotropinomas despite their high expression levels of sst5 and also sst2, and their clear response to octreotide, strongly suggest that additional mechanisms distinct from the mere presence of a given receptor are required for a drug to achieve its desired functional effect.

The sst expression profile observed in NFPAs (sst3>>sst2>sst5) is similar to that reported previously (Taboada et al. 2007), and suggested a possible responsiveness to pasireotide. However, only a small proportion of NFA was responsive, in vitro, to either SSA, at least in the parameters evaluated. Moreover, some of the effects observed could be considered paradoxical and, from a clinical perspective, unexplainable. Indeed, both SSA might increase cell viability compared with vehicle-treated cells in the majority of responsive tumors. Similar stimulatory actions of pasireotide (Zatelli et al. 2007) and of an sst5-specific agonist have been described previously in NFPAs, but the precise underlying mechanisms and specific ssts involved remain unclear. A reduction in cell viability can result from decreased cell growth and/or activation of apoptosis, whereas an increase of cell viability indicates that these compounds may protect primary cells from natural cell death and/or may activate survival/proliferative mechanisms. In the tumors examined, there were no statistical differences in sst expression profile between inhibitory responders and stimulatory responders. However, the fact that sst3 is uniquely expressed at high levels in NFPAs and that pasireotide seems more effective in stimulating cell viability in these tumors invites speculation that this receptor may be involved in such responses. Nevertheless, further studies...
are required to elucidate the precise role of sst3 and the rest of ssts in this unique response of NFPAs, which, already adds potentially useful information for the current understanding of SSA effects on these tumors. Actually, despite promising in vivo experiences with octreotide treatment in patients not cured after surgery (Fusco et al. 2012), which showed stabilized tumor size in most cases (but not tumor volume reduction), the poor in vitro response and the paradoxical increases in cell viability reported in response to both SSA discourages the use of SSA as first-line treatment in NFPAs.

Prolactinomas are often responsive to dopamine agonists. However, 10% of patients fail to normalize PRL levels and tumor growth (Fusco et al. 2008). The SSA therapy has been proposed as an alternative, since prolactinomas express ssts and several studies demonstrated that SSA are able to inhibit PRL release in primary cultures (Shimon et al. 1997, Hofland et al. 2004) and germane cell lines (Gruszka et al. 2007). Our results showed that octreotide decreased cell viability in one prolactinoma and only inhibited [Ca²⁺], in a very low proportion of cells, with pasireotide being even less effective despite high sst1 expression levels in these tumors. These results are in keeping with in vivo studies, which did not find consistent inhibitory effects (Fusco et al. 2011, Colao & Savastano 2011).

Finally, we also evaluated octreotide and pasireotide effects on a limited set of normal pituitary cell cultures. This revealed that, in line with in vivo analyses on healthy volunteers treated with octreotide (Tuvia et al. 2012) or pasireotide (Golor et al. 2012), where both SSA decreased hormone levels, particularly GH, both SSA directly acted on pituitary cells to decrease [Ca²⁺], kinetics. Additionally, we found that pasireotide, but not octreotide, also reduced cell viability in normal pituitary cell cultures.

In summary, our results indicate that octreotide and pasireotide act on the main types of pituitary adenomas by exerting similar and distinct effects on [Ca²⁺], kinetics, hormone release, gene expression and cell viability. However, we did not observe any evident correspondence between the effects observed and the specific sst1-sst5 profile of the target tumors. Hence, the emerging picture is that there might not be a simple predictive correspondence between the presence of a sole receptor and the response to a given SSA with high affinity for this receptor, but that other factors may substantially influence the response of pituitary tumor cells, such as the proportion of other ssts for which the SSA may not have high affinity, the signaling status of the target cell and so on. Therefore, further studies are warranted to better understand the functional actions of the two SSA investigated here as well as novel SSA, wherein the relevant endpoints and underlying mechanisms should be tested preferentially in the same ultimate targets for these drugs, that is, the primary pituitary tumor cells, as they may provide a more precise and realistic portrait of the actual response that can be expected, and, hopefully, predicted, for a given type of tumor.

Declaration of interest
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References


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