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Cell specific interaction of pasireotide: review of preclinical studies in somatotroph and corticotroph pituitary cells

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Abstract

Background Pasireotide is a second-generation somatostatin (SRIF) receptor ligand (SRL), approved for medical treatment of acromegaly and Cushing's disease (CD). The molecule is a stable cyclohexapeptide synthetized based on SRIF structure. Differently from first-generation SRLs (e.g. octreotide), preferentially binding somatostatin receptor (SST) subtype 2 (SST_2), pasireotide has high affinity for multiple SSTs ($SST_5 > SST_2 > SST_3 > SST_1$). Interestingly, early preclinical studies demonstrated that pasireotide shows distinct functional properties compared to SRIF and first-generation SRLs when binding SSTs.

Methods We aimed to highlight the differential receptor-targeted action of pasireotide in the treatment of somatotroph and corticotroph adenomas, throughout the critical revision of preclinical studies carried out on acromegaly and CD models.

Results Different authors demonstrated that the antisecretory effect of pasireotide in somatotroph adenoma cell cultures is comparable to that of the SST_2-preferential agonist octreotide. Some reports even show a direct correlation between SST_2 mRNA expression and GH reduction after pasireotide treatment, thus laying for a predominant role of SST_2 in driving pasireotide efficacy in somatotropinomas in vitro. On the other hand, the inhibitory effect of pasireotide on ACTH secretion in corticotropinoma cells seems to be mainly mediated by SST_5. Indeed, most reports show a higher potency and efficacy of pasireotide compared to SST_2 preferential agonists, while functional studies confirm the pivotal role of SST_5 targeting in corticotroph cells.

Conclusions The analysis of preclinical studies carried out in somatotroph and corticoph adenomas points out that pasireotide shows a cell-specific activity, exerting its biological effects via different SSTs in the different adenoma histotypes.

Keywords Pasireotide · GH-secreting · ACTH-secreting · Pituitary adenomas · Primary cultures

Introduction

Endogenous somatostatin (also known as somatotropin release-inhibiting factor, SRIF) shows a potent antisecretory effect in both physiological and pathological settings, as well as inhibitory effects on cell growth and differentiation [1]. Five functional main SRIF receptor subtypes (SSTs), including the splicing variants of SST_2 (SST_{2A} and SST_{2B}) plus two truncated isoforms of SST_5 (SST_5TMD4 and SST_5TMD5) have been cloned and characterized so far [2, 3]. Somatostatin receptors (SSTs) belong to a seven spanning transmembrane receptor superfamily, functionally coupled to G proteins, and mediate the multiple intracellular pathways activated by the binding of SRIF on cell membrane [4–8]. These receptors share common structural features and signaling properties, but differ in their cellular localization, trafficking, and regulation. Of note, a recent and extensive review article of the International Union of Basic and Clinical Pharmacology (IUPHAR) have carefully summarized the (patho)physiology and the pharmacology of SSTs [5].

Although endogenous SRIF shows a broad antisecretory activity on many hormones, due to its short half-life (< 3 min) and to the post-infusion rebound observed after treatment, it is unfitting the clinical practice [5, 9]. This issue prompted researchers to develop synthetic SRIF receptor ligands (SRLs) in order to overcome the above-mentioned limitations and become suitable for the clinical application.

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Octreotide and lanreotide represent the first-generation SRLs developed, tested and approved for the clinical use between the early 1980s' (octreotide synthesis) and 2001 (lanreotide Autogel formulation approved for acromegaly treatment) [10, 11]. Both compounds are small octapeptides, showing enhanced half-life, reduced clearance compared to native SRIF and, interestingly, a preferential binding affinity to SST_2 [12]. Because of the high expression of SST_2 detected in the majority of GH-secreting pituitary adenomas, first-generation SRLs currently represent the first line medical treatment for acromegaly [13, 14]. However, different SSTs are heterogeneously expressed in pituitary tumors. For example corticotroph adenomas, resulting in Cushing's Disease (CD), show a predominant expression of SST_5 [15, 16]. This condition led researchers to generate other compounds with a wider SST binding profile, possibly similar to endogenous SRIF [17].

In this context, pasireotide, also known as Signifor, has been synthetized based on SRIF structure and represents a second-generation SRL.

In detail, pasireotide is a stable cyclohexapeptide with a long half-life (~ 24 h) showing a binding affinity in the subnanomolar range for SST_5 (half-maximal effective concentration [EC50] 0.2 nM), and a reasonably high affinity for SST_1 (9.3 nM), SST_2 (1.0 nM) and SST_3 (1.5 nM) [5, 18]. However, despite the initial aim to search for a compound able to closely mimic native SRIF, early in vitro studies, investigating the biological mechanisms of pasireotide, demonstrated that this compound has distinct functional properties compared to both native SRIF and first-generation SRLs when binding SSTs, particularly SST_2 [19–21]. More in detail, when evaluating the typical signaling pathways activated by SRIF, a number of different authors defined pasireotide as a biased-agonist of SST_2. Indeed, Cescato and colleagues showed that pasireotide is a potent inhibitor of cAMP accumulation, although it antagonizes SRIF stimulation of intracellular calcium (another peculiar SST-mediated intracellular pathway) and behaves as partial agonists/antagonists for ERK phosphorylation [21]. Furthermore, another paper showed that pasireotide is less potent than SRIF (and octreotide) in the inhibition of cAMP levels and the activation of ERK1/2 pathway following SST_2 binding [19]. Interestingly, the same study highlighted pasireotide as a potent agonist of SST_5, comparable to the action of native SRIF and significantly more effective than octreotide [19]. Moreover, other studies demonstrated that pasireotide regulates SST_2 receptor trafficking differently than other ligands [20, 22, 23]. These authors showed that pasireotide treatment results in a reduced internalization and a faster recycling of SST_2 compared to both native SRIF and octreotide, inducing partial receptor phosphorylation and thus modulating the receptor/β-arrestin interaction in a peculiar manner (namely, less β-arrestin recruitment on cell membrane) [20, 22, 23].

However, despite the above mentioned studies highlight pasireotide as a biased agonist at SST_2 (while SST_5 seems to mediate most of its pharmacological actions), clinical experience on acromegaly and in vitro studies carried out on somatotroph adenoma primary cultures seem to point out a predominant role of SST_2 targeting in driving pasireotide efficacy in this pituitary adenoma hystotype.

In this light, a large clinical trial, conducted on medically naive acromegaly patients, compared the efficacy of pasireotide and octreotide in the normalization of biochemical parameters [24]. At the end of the study, patients treated with pasireotide had a significantly higher rate of normalized IGF-1 levels compared to those treated with octreotide. However, the effect of the two drugs in the normalization of circulating GH levels (defined as GH < 2.5 µg/L) was almost superimposable (48.3% and 51.6% for pasireotide and octreotide, respectively) [24]. The discrepancy observed between the inhibitory effect on IGF-1 and GH levels should be not underestimated, since this finding lays for a similar pituitary-targeted effect of the two compounds in the large cohort of unselected acromegaly patients analyzed in the study.

However, we face a completely different scenario when investigating the role of pasireotide in Cushing's Disease.

Indeed, clinical evidences show that octreotide does not have a suppressive action on ACTH and urinary free cortisol (UFC) in patients harboring a corticotroph adenoma [25–27], while pasireotide has been proven to be an effective tool in the treatment of CD, reducing both ACTH, plasma cortisol and UFC in about 20–30% of patients [28–30]. Therefore, SST_2-targeting via octreotide is not effective in CD patients, while the activity of pasireotide is likely to be mediated throughout SST_5 binding.

These data suggest that pasireotide could exert its biological function throughout the activation of different SST subtypes and/or intracellular pathways in somatotropinomas and corticotropinomas.

This manuscript aims to highlight the cell-specific activity of pasireotide in somatotroph and corticotroph adenoma cells through the activation of different SST subtypes in the two adenoma histotypes, as observed in a number of in vitro studies. Of note, a detailed description of the complex intracellular mechanisms involved in the biological response to pasireotide treatment in GH- and ACTH-secreting adenoma cell cultures is beyond the scope of this review. Indeed, a number of sub-cellular mechanisms are likely to be involved in these processes (e.g. differential receptor phosphorylation, desensitization, trafficking and receptor cross-talk) but a direct link between the described molecular events and the antisecretory efficacy of pasireotide in both somatotroph and corticotroph cell cultures (compared to first-generation SRLs) is still lacking and object of ongoing studies.

Pasireotide in preclinical models of acromegaly

The structure of pasireotide was built up using an "Ala-substituted" approach in order to identify the essential residues of native SRIF for the universal binding to human SST_{1-5} [31]. Since this very first study, pasireotide showed up as a potent inhibitor of GH secretion. Indeed, the compound was able to significantly reduce GH secretion (after GHRH stimulation) in primary cultures of rat anterior pituitary cells (65% inhibition after 3 h at 10 nM concentration). Interestingly, pasireotide was three- to four-fold time more potent compared to both SRIF-14 and octreotide, showing a half-maximal inhibitory concentration (IC50) in the sub-nanomolar range (0.4 ± 0.1 nM) [31]. Therefore, the same authors tested the efficacy of this novel molecule in the modulation of GH, IGF-1, insulin and glucagone levels in vivo (rat animal models), after both short- and long-term treatment (1 h to 126 days). After 1 h treatment, octreotide showed a slightly higher effect than pasireotide in reducing GH secretion (median effective dose [ED50] 0.13 vs 0.22 µg/kg), whereas, after prolonged treatment (6 h), pasireotide was more potent compared to octreotide (ED50 23.7 vs 5.5 µg/kg), demonstrating its prolonged biological action [31]. Noteworthy, after 126 days of continuous infusion at fixed dose (10 mg/kg/h), pasireotide also reduced IGF-1 levels by 75% compared with placebo-treated animals, while octreotide treatment resulted in only a 30% decrease of IGF-1 [31].

A following study by Murray and colleagues directly compared the effect of pasireotide, SRIF-14 and octreotide in reducing GH secretion in different in vitro models, such as primary cultures from rat pituitary cells, human fetal pituitary tissues and human GH-secreting pituitary adenomas [32]. Differently from the previous study of Bruns et al., the drug-comparison dose-dependent experiment carried out in rat cells showed no significant difference in GH inhibition between the two drugs after a 6 h treatment, with a computed IC50 for pasireotide and octreotide of 1.2 and 0.8 nM, respectively [32]. However, the same study aroused a great interest from a translational perspective. Indeed, the comparison between the effect of pasireotide, SRIF and octreotide (20 h treatment, 10 nM concentration) in six human somatotroph pituitary adenoma primary cultures, showed that pasireotide and SRIF-14 were able to inhibit GH release by more than 20% in 5 out of 6 samples, while octreotide induced a comparable percentage of GH inhibition only in 3 of 6 tumors. However, considering all adenoma primary cultures (n = 6), no difference in mean GH suppression was observed between pasireotide, SRIF-14 and octreotide treatment [32]. Since pasireotide and octreotide show a differential binding affinity for SSTs, the expression of the different subtypes was evaluated in the tumor samples by use of reverse transcriptase (RT)-PCR. All adenomas expressed SST_2 mRNA, while SST_5 was expressed in 4 out of 6 tumors. Interestingly, pasireotide inhibited GH secretion > 20% in the two SST_5-negative samples. These data suggested for the first time a prevalent role of SST_2, compared to SST_5, in driving pasireotide inhibitory effect on GH-secreting adenoma samples. However, due to the small sample size, no clear conclusions were drawn regarding the correlation between SST expression and pasireotide antisecretory activity.

The same year, Hofland and co-workers published an elegant study evaluating the efficacy of pasireotide, octreotide and SRIF-14 (72 h treatment, 10 nM concentration) on GH release in nine GH-secreting pituitary adenoma primary cultures [33]. Furthermore, the authors investigated SST expression on tumor samples (n = 7) using quantitative RT-PCR. The results showed that the percentage of GH suppression by the three compounds was comparable in most adenomas (all but one case). More in detail, all samples had a predominant, although variable, expression of SST_2 and SST_5 mRNA, and the authors found a direct and significant correlation between SST_2 mRNA expression and both octreotide and pasireotide antisecretory activity [33]. Of interest, the inhibitory effect of pasireotide did not correlate with SST_5 expression, despite the high binding affinity of the compound for this specific SST subtype [33]. Therefore, this finding highlighted the role of SST_2 receptor as the main target of pasireotide biological action on GH-secreting adenoma cell cultures.

More recently, Ibáñez-Costa et al. explored the molecular features associated with pasireotide response, compared to octreotide, in a larger number of somatotroph adenoma samples [34]. In a series of primary cultures from adenomas mainly expressing SST_5 and SST_2 ($SST_5 > SST_2$, mRNA evaluation by quantitative RT-PCR), pasireotide was as effective as octreotide in reducing GH secretion in the culture media at different time-points (4 and 24 h). Furthermore, the authors investigated the effect of pasireotide in the reduction of intracellular calcium concentration, $[Ca^{2+}]i$, a pivotal second messenger involved in the regulation of hormone secretion. In this context, the mean $[Ca^{2+}]i$ decreased after pasireotide and octreotide treatment was, once more, comparable, although the number of tumors responsive to treatment was higher after incubation with octreotide compared with pasireotide (15/21 vs 11/21).

In another study published in 2017, authors compared the antisecretory effect of pasireotide and octreotide on GH secretion in a large number of primary cultures of somatotroph adenomas (n = 33), and they correlated these data with the adenoma SST expression profile [35]. Due to the high number of samples included in the study, authors aimed to identify the presence of

peculiar adenoma subgroups in which the effect of one of the two compounds could be predominant [35]. Both compounds were tested "head-to-head" at the concentration of 10 nM for 72 h. In line with previous findings, pasireotide and octreotide equally reduced GH secretion (37.1% vs 36.8%, respectively). SST_2 mRNA expression significantly correlated with the efficacy of octreotide to reduce in vitro GH secretion, while a slight trend for linear correlation was observed for pasireotide as well. Conversely, SST_5 expression did not correlate with the effect of both compounds. Interestingly, six (18%) adenoma cultures showed a better response to pasireotide compared to octreotide (PAS + group), while five samples (15%) showed an opposite response to drug testing (OCT + group). PAS + adenoma group had significantly lower SST_2 levels compared with the other samples, as well as a lower SST_2/SST_5 ratio. However, the authors observed that tumor samples with a ≥ 50% GH inhibition after pasireotide treatment expressed significantly higher levels of SST_2 mRNA compared with tumor samples with a lower rate of GH reduction. Furthermore, the percentage GH decrease induced by pasireotide and octreotide treatment was strongly and directly correlated when performing a pairwise comparison of the different 33 adenoma cell cultures ($r = 0.829$, $p < 0.0001$), thus suggesting that, in most cases, the two compounds act via the same target receptor (namely, SST_2).

Table 1 summarizes the different preclinical studies evaluating the antisecretory effect of pasireotide vs octreotide in primary cultures of somatotroph adenoma and shows that the efficacy of the two compounds is comparable (almost superimposable). This finding is even more convincing when considering the different experimental settings of the various reports (e.g. incubation time from 4 to 72 h), carried out in different somatotroph adenoma cohorts.

As a consequence, and based on direct evidences laying for a positive correlation between SST_2 mRNA expression and pasireotide efficacy [33, 35], we suggest that in vitro data published so far highlight SST_2 as the main target receptor driving the antisecretory effect of pasireotide in somatotroph adenomas.

Table 1 In vitro studies comparing the efficacy of pasireotide (PAS) with the sst2-preferential agonist, octreotide (OCT), in human GH-secreting pituitary adenoma primary cultures

Study	Tumor tissue	Samples, n.	Measurement	PAS vs OCT effect	PAS vs OCT (overall efficacy)	SST expression	Notes
Murray et al. (2004) [32]	Somatotroph adenomas	6	GH secretion	34 ± 8% vs 18 ± 12% (Mean % inhibition)	PAS = OCT	$SST_2 > SST_5$ (mRNA, 6 vs 4 samples)	PAS effective (≥ 20%) in SST_5 negative samples
Hofland et al. (2004) [33]	Somatotroph adenomas	9	GH secretion	22 to 68% vs 26 to 73% (Range % inhibition)	PAS = OCT	$SST_5 > SST_2$ (mRNA, quantitative)	PAS efficacy directly correlates with SST_2 expression
Ibáñez-Costa A. et al. (2016) [34]	Somatotroph adenomas	7 (PAS) 9 (OCT)	GH secretion	~25%[a] vs ~25%[a] (Mean % inhibition)	PAS = OCT	$SST_5 > SST_2$ (mRNA, quantitative)	
		21	$[Ca^{2+}]i$	75 ± 2% vs 77 ± 2% (PMR, %)	PAS = OCT		
Gatto et al. (2017) [35]	Somatotroph adenomas	33	GH secretion	37.1 ± 16% vs 36.8 ± 16% (Mean % inhibition)	PAS = OCT	$SST_5 > SST_2$ (mRNA, quantitative; protein, semi-quantitative)	PAS effective (≥ 50%) with higher SST_2 levels PAS does not correlate with SST_5 expression

PAS pasireotide, *OCT* octreotide, $[Ca^{2+}]i$, intracellular calcium levels, *PMR* percentage of maximum response, = no statistically relevant difference

[a]Data extracted from graphs presented in the related article (namely, Fig. 2, Panel C, 24 h treatment)

Pasireotide in preclinical models of Cushing's disease

To date pasireotide is the only pituitary-targeted drug approved for medical treatment of CD by both EMA and FDA [30]. Similarly to the previously described role of octreotide and pasireotide in acromegaly, a number of preclinical studies investigated the biological effect of pasireotide in CD models before and after its approval for clinical use. The rationale for the use of pasireotide in CD resides in the high expression of SST_5 receptor ($SST_5 > SST_2$) unanimously reported in human corticotroph cells, as well as in other CD models (e.g. AtT20 cell line) [17, 36]. Furthermore, in vitro and ex vivo studies have shown that SST_5 mRNA expression is not affected by exposure to high glucocorticoid levels (the hallmark of active CD), whereas, the transcript of *SSTR2* gene is significantly downregulated [15, 25, 37–39].

A study by Hofland et al., published in 2005, directly compared the in vitro effect of pasireotide and octreotide on ACTH release by human corticotrophs and AtT20 cells [40]. Furthermore, the authors evaluated the SST mRNA expression in six adenoma samples. As expected, corticotroph adenoma cells predominantly expressed SST_5 mRNA ($> SST_2$), and pasireotide was more effective than octreotide in the inhibition of basal ACTH secretion (72 h treatment, 10 nM concentration). More in detail, pasireotide significantly suppressed ACTH secretion in 3 out of 5 primary cultures (− 30% to − 40%), while octreotide inhibited ACTH release in only 1 out of 5 cultures (28% suppression). To better elucidate the differences between pasireotide and octreotide, time-course and dose-dependent experiments were performed on AtT20 cells. In agreement with the above mentioned findings, pasireotide, but not octreotide, significantly suppressed basal ACTH secretion after 72 h, showing a higher potency (IC50 0.2 nM) compared to octreotide [40].

In line with the report from Hofland and colleagues, Batista et al. showed that pasireotide was able to inhibit ACTH secretion (max response: 48–96 h at 1–10 nM concentration) in 5 out of 6 corticotroph adenoma primary cultures (percent suppression: − 23 to − 56%) [41]. In this group of adenoma samples, a predominant expression of SST_5, compared to the other SST subtypes, was described at both mRNA and protein level (this latter evaluated by immunohistochemistry) [41].

A following study from van der Hoek et al. aimed to deeply investigate the differential properties of SST_5 and SST_2 in the regulation of ACTH release by corticotroph tumor cells [37]. At first, authors evaluated the inhibitory effect of pasireotide, SRIF-14, octreotide and BIM-23268 (an experimental SST_5-selective agonist) on CRH-induced ACTH release in AtT-20 cells in basal conditions (no glucocorticoid exposure), demonstrating a significantly more potent effect of pasireotide (IC50 0.06 nM) than octreotide (0.2 nM) in reducing ACTH levels into conditioned media. Furthermore, they pretreated AtT20 cells with 10 nM dexamethasone (DEX) for 48 h, showing that glucocorticoid exposure did not affect pasireotide effects, while it induced a 20-fold decrease of octreotide-induced inhibition of ACTH secretion (namely, IC50 from 0.2 to 4.3 nM). This finding was in line with the modulation of SST_2 mRNA expression observed after DEX treatment (− 45%), while SST_5 expression was not affected [37]. Interestingly, the inhibitory effect of BIM-23268 on CRH-induced ACTH release was significantly higher after DEX pretreatment compared to baseline (− 60% vs − 15%).

Since SST_5 expression seems not to be affected by DEX pretreatment, we could speculate that the modulation of the mutual membrane receptor interaction (e.g. SST_5–SST_2 crosstalk) and/or the DEX effect on the intracellular machinery might explain the enhanced activation of SST_5 antisecretory pathways after glucocorticoid exposure [17, 36, 42].

More recently, Ben-Shlomo and colleagues elegantly demonstrated that pasireotide mainly exerts its action via SST_5 activation in corticotroph cells [43]. The authors tested pasireotide, SRIF-14 and octreotide on AtT20 cells (wild-type or overexpressing human SST_2 [$hSST_2$] or SST_5 [$hSST_5$] receptors), alone or in combination with selective receptor antagonists. In wild-type (WT) cells, pasireotide was significantly more potent in the inhibition of cAMP compared to both SRIF-14 and octreotide (IC50 55 pM vs 370 pM vs 470 pM, respectively). Accordingly, pasireotide was more effective than octreotide in suppressing the normalized calcium oscillation average (NOA), as well.

Differently from octreotide, pasireotide was less potent in reducing cAMP levels in cells stably transfected with $hSST_2$, compared to WT-cells. Furthermore, co-incubation with an SST_2-selective antagonist (BIM-23454) did not affect pasireotide potency or efficacy to reduce cAMP in WT-AtT20 cells. Therefore, the SST_2-mediated activation of pasireotide in AtT20 cells seems to be negligible, at least at therapeutic doses [43]. On the other hand, $hSST_5$ overexpression prevented pasireotide desensitization, thus resulting in a long-term increased potency of the compound. Although $hSST_5$ overexpression in AtT20 cells did not directly enhance pasireotide potency (IC50 already in the picomoles range in the WT-setting), the authors concluded that SST_5 is the major determinant of pasireotide inhibitory effect on cAMP in AtT20 cells.

Interestingly, in 2011 Castillo et al. showed that pasireotide-mediated pro-opiomelanocortin (POMC) promoter activity in AtT20 cells was reduced via SST_2 (through G_i α-subunit) [44]. Indeed, the effect of pasireotide in the inhibition of POMC relative luciferase activity was completely

abolished in SST_2-knockdown cells, while the effect of the compound did not change when treating cells transfected with SST_5 siRNA [44]. In line with this finding, Murasawa and colleagues demonstrated that pasireotide-mediated inhibition on POMC mRNA expression in AtT20 cells was not affected by SST_5 downregulation [45]. Therefore, the effect of pasireotide on POMC mRNA expression in AtT20 cells seems not mediated by SST_5 activation.

Focusing on the results on POMC promoter activity and POMC mRNA expression showed by Castillo and Murasawa, these data seem to be in contrast with previous reports [37, 40, 43]. Indeed, pasireotide-mediated inhibition of POMC appears to be mainly dependent on SST_2 activation, while ACTH synthesis and secretion (supposed to be correlated with POMC expression) is mainly associated to SST_5 activation by pasireotide in human corticotroph adenoma cells and AtT20 cell line.

Therefore, based on current evidences, the path to uncover the peculiar biological activity of pasireotide in corticotroph adenoma cells is not straightforward. For instance, Ibáñez-Costa et al. recently found that octreotide was more effective than pasireotide in the inhibition of ACTH release in human corticotroph adenoma primary cultures (100 nM, 24 h treatment) [34]. More in detail, high dose of octreotide significantly reduced ACTH-secretion in 2 out of 3 tumor samples analyzed, while the same concentration of pasireotide only induced a slight ACTH reduction in 2 of 4 tumors [34]. Furthermore, the same authors showed that both compounds were able to decrease [Ca2+]i kinetics in about 50% of corticotroph adenomas (4/10 and 5/9, respectively), but pasireotide was significantly less effective than octreotide, thus resulting in a lower degree of PRC (proportion of responsive cells; 12.9% vs 56.1%) [34]. Interestingly, in line with previous findings, the corticotroph adenoma samples included in this study showed a clear predominant expression of SST_5 compared to the other SSTs (evaluated as mRNA levels) [34].

In our opinion, the use of high dose of octreotide in this experimental setting could partially explain the observed efficacy of this compound, based on a possible activation of SST_5 (beyond SST_2) in corticotroph cells when testing supra-physiological concentrations (tenfold higher to the assumed therapeutic dose).

On the other hand, authors themselves suggested that a recovery of SST_2-related responsiveness could be due to the fact that the great majority of surgical specimens analyzed in their study were obtained from patients with CD treated with medical therapy before surgery, thus resulting in an increased SST_2 mRNA expression. However, no clear information about cortisol levels in patients at time of pituitary surgery was provided [34]. Furthermore, differently from the previous study of Hofland et al. [40], the comparison between pasireotide and octreotide inhibitory effect on ACTH secretion was not performed in the setting of a "head-to-head" study design (direct comparison of two compounds in the same cell cultures), thus limiting the strength of the results. Indeed, this point could raise some issues related to the well-known heterogeneity of corticotroph adenomas, possibly affecting the responsiveness to medical treatment [30, 46, 47].

In this light, van der Pas and colleagues directly compared the efficacy of pasireotide and octreotide in reducing ACTH secretion (10 nM concentration, 72 h treatment) in four primary cultures obtained from patients showing normal urinary free cortisol (UFC) levels before adenomectomy [15]. Of note, all samples showed a predominant expression of SST_2 compared to SST_5 mRNA, whereas SST_5 protein expression was higher than SST_2 in 2 out of 4 tissues.

In this context, pasireotide was significantly more effective than octreotide in 3 out of 4 samples (overall mean percent reduction: 49% vs 26%, respectively). Furthermore, a dose–response experiment (1 pM to 1 μM, 144 h treatment) was performed in tumor cells obtained from a patient nearly reaching normal UFC before surgery (UFC 1.06 × ULN). Interestingly, pasireotide showed an IC50 of 0.2 nM whereas octreotide significantly decreased ACTH secretion only at relatively high doses (IC50 39 nM) [15].

These results strongly support the significant inhibitory effect of pasireotide on ACTH secretion in human corticotroph adenoma cells in vitro, as previously reported by both Hofland [40] and Batista [41] (see Table 2). Moreover, the results from van der Pas et al. strength the hypothesis of a major role of SST_5 in driving the antisecretory activity of pasireotide in ACTH-secreting pituitary adenomas. Indeed, pasireotide is more effective than octreotide even in presence of a higher expression of SST_2 mRNA in tumor cells compared to SST_5 [15].

Finally, as observed in the in vitro studies evaluating the role of pasireotide in acromegaly, also preclinical studies on pasireotide and CD (above summarized and analyzed) show a high heterogeneity in the study design, particularly in respect to time (24–144 h) and dose (1 pM to 100 nM) of treatment. Of course, this issue makes more difficult the aim to draw consistent conclusions from an already complex scenario. However, based on preclinical evidences published so far, we can reasonably state that the antisecretory effect of pasireotide on ACTH secretion from human corticotroph adenomas, as well as mouse AtT20 cell line, is mainly driven by SST_5 binding and activation.

Conclusions

Pasireotide is a second-generation SRL, originally synthetized to mimic the biological properties of native SRIF [31]. However, a number of studies have clearly demonstrated

Table 2 In vitro studies evaluating the efficacy of pasireotide (PAS), alone or compared to octreotide (OCT), in human ACTH-secreting pituitary adenoma primary cultures

Study	Tumor tissue	Samples, n.	Measurement	PAS vs OCT effect	PAS vs OCT (overall efficacy)	SST expression	Notes
Hofland et al. (2005) [40]	Corticotroph adenomas	5	ACTH secretion	30–40% (n = 3/5) vs 28% (n = 1/5) (Range % inhibition)	PAS > OCT	$SST_5 > SST_2$ (mRNA, quantitative)	Dex pre-treatment did not reduce the inhibitory effect of PAS (One sample tested)
Batista et al. (2006) [41]	Corticotroph adenomas	6	ACTH secretion	23–56% (Range % inhibition)	n.a.	$SST_5 > SST_2$ (mRNA, quantitative; protein, semi-quantitative)	Statistically significant effect of PAS in 5 out of 6 primary cultures
van der Pas et al. (2013) [15]	Corticotroph adenomas	4	ACTH secretion	49 ± 5% vs 26 ± 7% (Mean % inhibition)	PAS > OCT	$SST_5 < SST_2$ (mRNA, quantitative) $SST_5 > SST_2$ (protein, semi-quantitative)	Primary cultures from patients with normal UFC levels before surgery
Ibáñez-Costa et al. (2016) [34]	Corticotroph adenomas	4 (PAS) 3 (OCT)	ACTH secretion	~10%[a] vs ~25%[a] (Mean % inhibition)	PAS < OCT	$SST_5 > SST_2$ (mRNA, quantitative)	High dose of PAS and OCT tested (100 nM)
		9 (PAS) 10 (OCT)	$[Ca^{2+}]i$	83 ± 2% vs 69 ± 6% (PMR, %)	PAS < OCT		

PAS pasireotide, *OCT* octreotide, > more effective, < less effective, *UFC* urinary free cortisol, *Dex* dexamethasone, $[Ca^{2+}]i$ intracellular calcium levels, *PMR* percentage of maximum response

[a]Data extracted from graphs presented in the related article (namely, Fig. 3, Panel C, 24 h treatment)

that, despite a broad spectrum of binding affinity for different SST subtypes, pasireotide is able to activate peculiar intracellular pathways and to modulate receptor internalization/trafficking differently from the other SRLs and from native SRIF as well. These findings shed light on the important role played by the whole intracellular machinery involved in the activation of the second messenger cascade beyond the mere concept of binding affinity (the higher affinity, the greater efficacy) [36, 48, 49]. In this context, the analysis of the effects of pasireotide, alone or compared to first-generation SRLs, in preclinical models of acromegaly and CD, has led to uncover a cell-specific activity of the compound in these different adenoma histotypes (Fig. 1). Indeed, as discussed throughout the manuscript, in GH-secreting pituitary adenomas strong evidences indicate that, overall, pasireotide exerts its antisecretory activity mainly by the activation of SST_2. This finding is not in contrast with the hypothesis that, in selected cohorts of somatotroph adenomas, resistant to first-generation SRLs and showing very low SST_2 expression, pasireotide could also exert its functions via SST_5 binding [35, 50]. From a translational perspective we could speculate that, based on the classification of acromegaly proposed by Cuevas-Ramos et al., pasireotide mainly acts via SST_2 in type 1 disease (good response to first-generation SRLs, high SST_2 expression), while its effects are mediated (also) via SST_5 in type 2 and type 3 acromegaly (unsatisfactory response to first-generation SRLs, low SST_2 expression) [51].

On the other hand, the insights from preclinical studies on CD lay for a predominant role of SST_5 as target receptor for pasireotide biological effects (particularly, the inhibition of ACTH secretion and cAMP levels). This evidence is mainly supported by the direct comparison of pasireotide effects with first-generation SRLs (e.g. octreotide) in corticotroph adenoma primary cultures, as well as in the murine corticotroph cell line AtT20. In this setting, pasireotide is more potent and effective than a preferential SST_2-agonist (e.g. octreotide) in inhibiting ACTH-secretion and reducing intracellular cAMP levels [15, 37, 40, 43]. In the light of the complex scenario of corticotroph adenomas, our conclusions drawn from in vitro evidences are (indirectly) supported by clinical studies reporting the lack of efficacy of first-generation SRLs in reducing ACTH production and secretion in patients with CD.

We want to highlight that most of preclinical studies reviewed in the present manuscript correlated the efficacy/

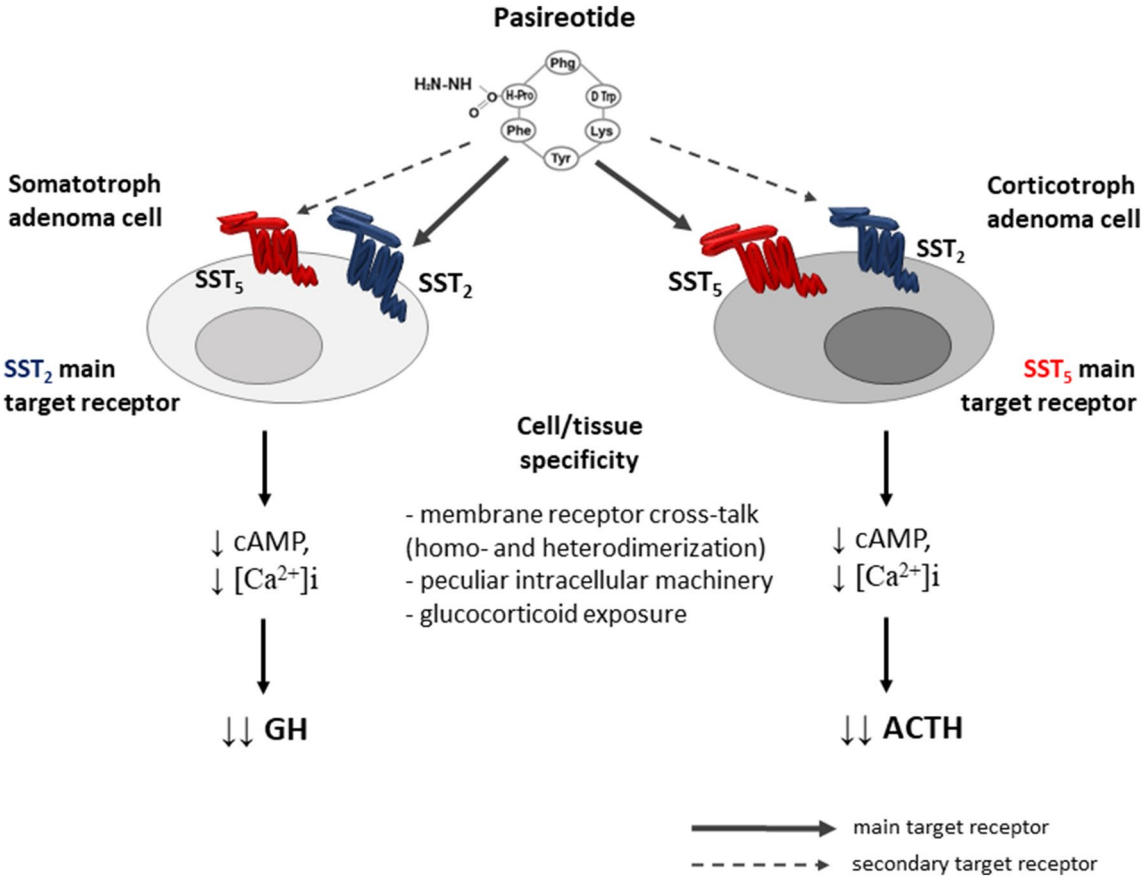

Fig. 1 Schematic representation of the differential receptor-targeted action of pasireotide in somatotroph and corticotroph adenoma cells. Overall, the antisecretory activity of pasireotide seems to be mainly driven by SST_2 in somatotroph adenomas, while SST_5 plays a major role in corticotroph pituitary adenomas. *ACTH* adrenocorticotropic hormone, *cAMP* cyclic adenosine monophosphate, [Ca2+]*i* intracellular calcium concentration, ↓ reduction, *GH* growth hormone, *SST* somatostatin receptor subtype

potency of pasireotide to the SST expression evaluated at mRNA level. Indeed, we are aware that SST mRNA and protein expression (this latter evaluated with immunohistochemistry) may not correlate [52]. However, as previously reported, the evaluation of SST mRNA expression by (quantitative) RT-PCR, directly performed on the same dispersed adenoma cells afterward plated to evaluate the in vitro effect of the tested compounds, can provide clearer indications about the role of the different receptors, compared with the immunohistochemical evaluation on paraffin-embedded tissues [35]. Of course, we think this is not the case when analyzing the role of SSTs in driving the in vivo responsiveness to SRLs (e.g. SST_2 immunoreactivity of somatotroph adenomas directly correlates with treatment response to first-generation SRLs in acromegaly patients) [53–55].

In conclusion, a critical analysis of preclinical studies carried out in acromegaly and CD models point out that the SST pan ligand pasireotide shows a cell-specific activity in somatotroph and corticoph adenoma cells, exerting its biological effects via different SST subtypes in the different adenoma histotypes.

This finding needs to be carefully considered when looking to the results of translational and clinical studies in acromegaly and CD, in order to identify the best positioning of pasireotide in the different treatment algorithms, as well as to optimize its efficacy in the specific clinical settings.

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Compliance with ethical standards

Conflict of interest FG has been a speaker for Novartis and has participated on advisory boards of Novartis, AMCo Ltd, and IONIS Pharmaceuticals. DF has been a speaker for and participated on advisory boards and received research grants from Novartis, Ipsen and Pfizer. The other Authors have no conflicts of interest to declare.

Research involving human and animal rights This article does not contain any studies with animals performed by any of the authors.

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