Control of pituitary adenoma cell proliferation by somatostatin analogs, dopamine agonists and novel chimeric compounds

Maria Chiara Zatelli, Maria Rosaria Ambrosio, Marta Bondanelli and Ettore C degli Uberti

Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies, University of Ferrara, Via Savonarola 9, 44100 Ferrara, Italy

(Correspondence should be addressed to M C Zatelli; Email: ztlmch@unife.it)

Abstract

The antisecretory effects of somatostatin (SRIH) and its analogs are widely recognized and provide the basis for treatment of hormonal hypersecretion in patients with pituitary adenomas, especially in the settings of acromegaly. Dopamine (DA) agonists have also been used for medical treatment of prolactin and/or GH hypersecretion, and recent evidence points to an even greater antisecretory effect for a chimeric molecule, having high affinity for both SRIH and DA receptors. Evidence for an antiproliferative effect of these compounds has also been provided. This review focuses on the antiproliferative effects of SRIH and its analogs, of DA and chimeric compounds on pituitary adenomas, and on the clinical consequences on tumor volume of pharmacological treatment of pituitary adenomas with these drugs.

European Journal of Endocrinology **156** S29–S35

Since the discovery of the antisecretory properties of somatostatin (SRIH) analogs, these compounds have been widely employed in the medical therapy of pituitary adenomas. The natural peptide, SRIH, is secreted by hypothalamic neurons into the hypophyseal portal circulation and regulates pituitary hormone secretion (1). It inhibits growth hormone (GH) and thyroid-stimulating hormone secretion (2), and also prolactin (PRL) and adrenocorticotropic hormone (ACTH) secretion under some circumstances (3). SRIH transduces its inhibitory effects by interacting with five different somatostatin receptor (SSTR) subtypes, in normal pituitary cells as well as in pituitary adenomas (4). The evidence that SRIH and its analogs have antisecretory effects prompted many studies aimed at investigating the possible antiproliferative effects of these compounds on pituitary adenomas. Indeed, it has been demonstrated that native SRIH inhibits the proliferation of both normal and neoplasic cells by inducing cell cycle arrest or apoptosis, depending on the receptor subtype and the target tissue (5–7). As a consequence, many studies have been performed and octreotide, one of the first SRIH analogs being synthesized and used in clinical practice, has been demonstrated to significantly reduce not only PRL secretion, but also DNA synthesis in a rat prolactinoma cell line (8). Moreover, in these settings, evidence for desensitization was shown due to loss of SSTR expression, which was restored by withdrawal from long-term exposure to octreotide. In addition, a dissociation between antiproliferative effects of octreotide, being apparent when SSTRs are highly expressed, and antisecretory effects, appearing for much lower SSTR expression levels in the same rat prolactinoma cell line, was suggested (9). Therefore, many studies subsequently explored SSTR subtype expression in pituitary adenomas, demonstrating that SSTR2 is the most frequently expressed receptor (10). These studies provided the basis for the use of SRIH analogs in the medical therapy of pituitary adenomas, in the attempt to restrain pituitary tumor growth and function. Evidence for octreotide's directly antiproliferative effects was provided by a multicenter randomized trial recruiting 32 acromegalic patients with pituitary macroadenoma. A group of 16 patients underwent octreotide treatment for 4 months before surgical resection, showing a 83% reduction in the mean growth fraction in comparison with the untreated surgical controls (11), indicating that octreotide pretreatment significantly suppressed pituitary adenoma cell growth. In addition, another study showed that apoptosis was not significantly altered by the octreotide pretreatment (12), suggesting that a reduction in cell cycling, rather than an increase in apoptotic rate, is the basis for the antiproliferative action of octreotide in somatotrope adenomas. This evidence was further confirmed by a clinical study where 14 acromegalic patients underwent a 3-month

pretreatment with SRIH analogs, showing more prominent regressive changes and increased perivascular fibrosis when compared with 17 surgical controls. Moreover, the Ki-67 labeling index was lower in treated than in untreated patients, supporting the hypothesis that SRIH analogs have antiproliferative effects on somatotrope adenomas (13). These effects, however, are not mirrored by significant morphological changes, since octreotide pretreatment does not cause necrosis, whereas it induces acidophilia and interstitial fibrosis, with a slight reduction in cell size (14). This picture greatly differs from that observed in prolactinomas after treatment with a dopamine (DA) agonist, where marked reduction in cellular, cytoplasmic and nuclear volume as well as marked perivascular and interstitial fibrosis and effects on the endoplasmic reticulum and Golgi have been observed (15). A recent review summarized the results of SRIH analog treatment on the size of pituitary adenomas in 921 acromegalic patients (16), showing that 42% of the patients displayed a reduction in tumor volume. This effect was more evident in patients in primary therapy (52%), when compared with patients who had already undergone surgery (21%). In addition, only 5% of the patients experienced further tumor enlargement, suggesting that treatment with SRIH analogs may contribute to the control of tumor growth in the majority of patients, at least for the treatment periods of up to 3 years.

The *in vivo* antiproliferative effects of octreotide are in agreement with previous *in vitro* studies, showing a significant reduction in cell number in three out of six primary cultures from GH-secreting pituitary adenomas exposed for 3 days to 1–10 μM octreotide (17). Similar effects were shown in primary cultures from clinically nonfunctioning pituitary adenomas (NFA), where significant inhibition of cell number was obtained for higher octreotide concentrations (10 μM), suggesting a lower SSTR expression level in these pituitary tumors (17). More insights into the antiproliferative effects of SRIH and its analogs have been provided by a more recent study analyzing 15 GH-secreting pituitary adenomas. SSTR2 and SSTR5 mRNAs were expressed in all tumors, while SSTR1 and SSTR3 mRNAs were expressed in 11 and 12 tumors respectively. Primary cultures were performed and tested with 1 μM SRIH or lanreotide (Lan), a SRIH analog interacting with both SSTR2 and SSTR5. After 24 h, no effect was observed in some cultures, but in others (as well as in the GH3 rat cell line) a parallel reduction in cell viability and DNA synthesis was observed. Moreover, tumors that were responsive to SSTR2- and SSTR5-specific analogs were also responsive to Lan. This study also highlighted a dissociation between the *in vitro* effects of SRIH analogs on tumor cell proliferation and GH secretion, suggesting that the inhibition of cell proliferation occurs independently of the effects on GH secretion (18).

The mechanism by which SRIH inhibits pituitary cell proliferation has been investigated in the GH3 rat

pituitary cell line, where octreotide and a chimeric compound, formed by GH-releasing peptide linked to SRIH, significantly inhibited cell proliferation by activating protein tyrosine phosphatases (PTP), involving a cGMP-dependent pathway (19). The involvement of PTPs was then confirmed by further studies performed in GH-secreting pituitary adenoma primary cultures, where SRIH and Lan inhibited cell proliferation induced by phorbol esters (PMA). This inhibitory effect was blocked by incubation with vanadate, a nonspecific PTP inhibitor, suggesting that this enzyme family is involved in mediating the antiproliferative effects of SRIH and its analogs. Moreover, this study showed that the inhibition of GH secretion was mediated by blockade of calcium channels and adenylyl cyclase activity, further supporting the hypothesis that the inhibition of cell proliferation occurs independently of the effects on GH-secretory pathways (20). It has been recently demonstrated that octreotide can induce a dose-dependent increase in caspase-3 activity, indicating that this compound can promote apoptosis in somatotrope tumors. A similar effect was also observed after treatment with a selective SSTR2 agonist, BIM-23120, which induces apoptosis by a phosphatase-dependent mechanism and causes growth arrest by an increase in p27 and a decrease in cyclin D1 expression (21). This finding confirmed the previous studies by Luciani *et al.* (22), showing that octreotide treatment induces apoptosis in GH-secreting adenomas, where the expression level of the antiapoptotic gene seladin-1 is significantly lower when compared with NFA. In addition, SRIH analogs have been shown to produce their antiproliferative action by acting on the PI3K/Akt signaling pathway and increasing *Zac1* gene expression. Zac1 induces cell cycle arrest and apoptosis and is highly expressed in normal pituitary, mammary and ovarian glands but is downregulated in pituitary, breast and ovarian tumors, suggesting that it might act as an oncosuppressor gene (23).

Previous studies demonstrated SSTR expression in a group of NFA, where SRIH and Lan inhibited PMA-stimulated cell proliferation in most NFA primary cultures. This effect was once again blocked by vanadate incubation, providing further evidence for PTP involvement in SRIH signal transduction in pituitary adenomas (24). Indeed, PTPs are listed among the pathways activated by SSTRs triggering cell proliferation inhibition, since the phosphatase family, and especially SHP-1, is involved in the control of apoptosis and in cell cycle regulation (25). We have previously demonstrated that constitutive SHP-1 activity controls cell proliferation in a human medullary thyroid carcinoma cell line, providing also evidence that, upon SSTR2 activation, SHP-1 activity can be further upregulated leading to cell proliferation inhibition, despite constitutive activation of potent proliferative stimuli (26). Taken together, these results not only confirm that SSTR2 is important in transducing the antiproliferative signals of SRIH, but

Table 1 *In vitro* antiproliferative effects of SRIF analogs and dopamine (DA) agonists in pituitary tumors.

Author (year)	Reference number	Disease	Sample number	Treatment	Results
Renner (1994)	(17)	Acromegaly	6	1–10 μM octreotide	Reduction in cell number in three cultures
Danila (2001)	(18)	Acromegaly	15	SRIH-, Lan-, SSTR2-, and SSTR5-selective agonists at 1 μM	Proliferation reduction of 5–60% in 15–40% of the cases
Florio (2003)	(20)	Acromegaly	13	SRIH, Lan at 1 μM	Reduction in PMA-induced cell proliferation in ten adenomas by 50–70%
Zatelli (2003)	(29)	Acromegaly	15	SRIH- and a SSTR1-selective agonists at 10 nM	Reduction in cell viability by 17.5–20%
Renner (1994)	(17)	NFA	13	Octreotide at 1–10 μM	Reduction in cell number in six cultures
Florio (1999)	(24)	NFA	23	SRIH and Lan at 1 μM	Variable inhibition of PMA-induced cell proliferation
Renner (1994)	(17)	NFA	13	Bromocriptine 1 μM 10 μM	15–29% cell number reduction 13–43% cell number reduction
Zatelli (2004)	(31)	NFA	12	SRIH- and a SSTR1-selective agonists at 10 nM	Reduction in cell viability by 17.5–20%
Gruszka (2006)	(62)	NFA	10	SSTR/DA chimera Bromocriptine	24% cell viability reduction 41% cell viability reduction
Sarkar (2005)	(46)	Rat pituitaries		Bromocriptine 1 μM	Reduction in proliferating lactotrope by 10%
Ferrante (2006)	(21)	Acromegaly	8	Octreotide 10 nM Octreotide 0.1 nM	160% increase in caspase-3 activity 172% increase in cleaved cytokeratin 18
Luciani (2005)	(22)	Acromegaly	5	Octreotide 10 nM	150% increase in cleaved cytokeratin 18
Theodoropoulou (2006)	(23)	Acromegaly (GH3 cell line)		Octreotide 1–100 nM	Increase in *Zac1* gene expression Decrease in PI3K tyrosine phosphorylation levels Increase in dephosphorylation Akt and GSKB
Batista (2006)	(61)	Cushing's disease	6	SOM230	10–70% cell viability reduction

also suggest that more than one SSTR is able to induce antiproliferative effects. At the same time, multiple subtypes may cooperate to induce growth arrest, also in the light of the recent advances on SSTR biology, indicating the possibility for these receptors to homo- and heterodimerize with other G-protein coupled receptors (27). The availability of SSTR-selective analogs therefore provides the opportunity to study the effects of activating single versus multiple SSTRs, since it now appears that the concept of a single dominant SSTR subtype controlling a specific biological function is too simplistic (28).

Trying to elucidate which other SSTR might be involved in transducing SRIH antiproliferative effects, we investigated SSTR1 activation in pituitary adenomas. For this purpose, we selected a group of 15 GH-secreting pituitary adenomas expressing SSTR1 and performed primary cultures that underwent treatment with SRIH or with a SSTR1-selective agonist, BIM-23926 at 10 nM. As expected, we observed a significant inhibition in GH and PRL secretion not only under SRIH treatment, but also under treatment with the SSTR1-selective agonist. Interestingly, this compound was as potent as SRIH in

inhibiting pituitary cell viability *in vitro*. Moreover, we found a significant positive correlation between the antiproliferative effects of BIM-23926 on GH and PRL secretion and SSTR1 mRNA expression. However, such correlation was not evident for the antiproliferative effect, suggesting that even very low SSTR1 expression might induce the antiproliferative effects of the SSTR1-selective agonist (29).

These results also indicate that SSTR1 may mediate SRIH actions at pituitary level, suggesting that this receptor may represent a target for new therapies attempting to control growth and hormonal secretion of pituitary tumors. This hypothesis is further supported by the finding that a SSTR1-selective agonist significantly inhibits GH secretion *in vitro* also in tumors from patients resistant to octreotide or Lan therapy (30).

The importance of SSTR1 as a possible target for medical therapy of pituitary adenomas was further underlined by the study we performed on a selected group of 12 NFA, all expressing SSTR1, SSTR2 and SSTR5, as well as chromogranin A (CgA) and α-subunit. We showed that SSTR1- and SSTR2-selective agonists inhibit both α-subunit and CgA secretion by

NFA primary cultures. Moreover, we showed that only the SSTR1-selective agonist was capable of significantly reducing *in vitro* cell viability, while the SSTR5-selective agonist enhanced pituitary cell viability. Again, the inhibitory effects of the SSTR1-selective agonist did not correlate with the extent of SSTR1 expression, further supporting the hypothesis that even very low SSTR1 expression might induce the inhibitory effects of the SSTR1-selective agonist (31). These results suggest that SRIH analogs with enhanced potency for SSTR1 could offer useful tools for the medical therapy of NFA. To the contrary, our results show that the treatment with a SSTR5-selective agonist significantly promoted NFA cell viability. This effect might account for the lack of NFA volume reduction observed in some patients treated with octreotide or Lan, which mainly interact not only with SSTR2, but also with SSTR5 (32–35). Moreover, our findings could explain the increase in tumor volume observed in some cases during such therapy (33), suggesting that SSTR5-interacting drugs might fail to control NFA tumor growth or, on the contrary, might facilitate pituitary cell survival in these adenomas. Therefore, the indication for medical therapy with octreotide or Lan in NFA patients with evidence for tumor SSTR5 expression should be carefully evaluated. On the other hand, it has been demonstrated that growth reduction of the mouse thyrotrope tumor cell line TtT-97 by thyroid hormone treatment is associated with increased expression of SSTR5 (36). Further experiments led to the hypothesis that thyroid hormones, acting through increased SSTR5, could activate the ERK pathway, resulting in a reduced expression of critical cell cycle proteins and a subsequent arrest of S-phase progression. These processes were reversed upon withdrawal of thyroid hormones, resulting in an increase in the fraction of S-phase cells (37). In addition, it has been recently demonstrated that treatment of dispersed GH-secreting pituitary adenoma cells with octreotide, as well as pasireotide (SOM230, a novel SRIH analog interacting with all SSTRs except for SSTR4), upregulates the expression of a cyclin-dependent kinase inhibitor, p27, possibly resulting in cell cycle inhibition (38).

Besides the direct actions on pituitary cells, SRIH and its analogs also have indirect effects, since they inhibit production and secretion of many angiogenic factors, therefore reducing tumor growth rate (39). Neoangiogenesis is fundamental for tumor growth and one of the main factors promoting this process is vascular endothelial growth factor (VEGF). It has been recently demonstrated that VEGF is overexpressed in the pituitary of DA receptor subtype 2 (DR2) knockout mice (40), indicating that this growth factor is under dopaminergic control. DR2 is extensively expressed in all pituitary tumor types, as demonstrated by *in situ* hybridization and immunohistochemistry (41). It has also been demonstrated that DR2 knockout mice develop prolactinomas with age (42, 43), DA

transporter gene knockout mice show arrested lactotrope development (44), and transgenic mice bearing the short isoform of DR2 (DR2s) display lactotrope hypoplasia (45). This evidence provides the basis for the use of DA agonists in the medical treatment of prolactinomas and highlights the importance of DA agonists in the control of lactotrope proliferation. Indeed, it has been recently demonstrated *in vivo* and *in vitro* that lactotrope treatment with DA and its agonist, bromocriptine, induces transforming growth factor (TGF)-β1 expression and secretion, which, in turn, can mediate the antiproliferative effects of DA on lactotropes. In addition, constitutive expression of the DR2s receptor upregulated the TGFβ1 expression and action, and inhibited cell proliferation in transformed lactotropes (46). DR2 activation in lactotropes leads to the alteration of G-protein coupling, inhibition of adenylyl cyclase, and reduction of intracellular cAMP (47, 48). In addition, DRs have been shown to regulate other transduction pathways that lead to alteration of intracellular calcium, protein kinase C, and the MAPK pathway (45, 47–50), resulting in reduced cell proliferation in a number of cellular models.

However, the effects of DA agonists on pituitary tumors different from prolactinomas are still a matter of debate. Indeed, some clinical studies showed that treatment with DA agonists has no effect on NFA volume (51), while others documented tumor shrinkage of up to 11% (52). *In vitro* studies have shown that, depending on the dose, bromocriptine is capable of significantly reducing the cell number in NFA, an effect reversed by a DR2 antagonist, haloperidol (17). Further studies showed that DR2 expression is variable in NFA, and that recurrent tumors lack expression of DR2s (53). Moreover, three out of nine NFA cultures responded to bromocriptine in terms of DNA synthesis reduction, and all responder adenomas expressed DR2s. In all cases, haloperidol, a DR2-selective antagonist, blocked the inhibitory effects of bromocriptine, demonstrating that this action was mediated by DR2. These data suggest that DR2s expression in NFA favors the growth suppressive effect of bromocriptine. This hypothesis is further supported by the finding that DR2s expression is lower in resistant prolactinomas when compared with responsive adenomas (54), and that increased DR2s expression in lactotrope cells results in pituitary hypoplasia (45). The mechanisms underlying the antiproliferative effects of DA agonists have been explored in the GH3 cell line, where the specific inhibitors of p38 MAPK and ERK pathways completely abrogated such effect, indicating that DA induces antiproliferative effects and cell death via DR2, involving p38 MAPK and ERK pathways (55).

Moreover, it has been recently demonstrated that approximately 80% of corticotrope pituitary tumors express functional DR2 and that treatment with cabergoline is effective in controlling the cortisol hypersecretion associated with Cushing's disease (56).

Table 2 *In vivo* antiproliferative effects of SRIF analogs and DA agonists in pituitary tumors.

Author (year)	Reference number	Disease	Sample number	Treatment	Results
Thapar (1997)	(11)	Acromegaly	36	Octreotide s.c. (4 months)	83% reduction in growth fraction
Losa (2001)	(12)	Acromegaly	39	Octreotide s.c. (12 months)	53% reduction in ki67 labeling index
Cap (2003)	(13)	Acromegaly	14	Octreotide (13 pts) and lanreotide (1 pt; 3 months)	67% reduction in ki67 labeling index
Bevan (2005)	(16)	Acromegaly	921	Various compounds and treatment length	Various extents in tumor size reduction in 42% of pts
Hubina (2006)	(38)	Acromegaly	25	Octreotide (12 months)	p27 upregulation
Oppizzi (1998)	(32)	NFA	22	Octreotide	No changes in tumor volume
Andersen (2001)	(33)	NFA	10	Octreotide and cabergoline	30% reduction in tumor volume in 6 pts
Gasperi (1993)	(35)	NFA	8	Octreotide s.c. (6 months)	No changes in tumor volume
Bevan (1986)	(51)	NFA	20	Bromocriptine	No changes in tumor volume
Liuzzi (1996)	(52)	NFA	n.d.	DA agonists	11% reduction in tumor volume
Pivonello (1999)	(56)	Nelson's syndrome	1	Cabergoline (6 months)	Disappearance of pituitary microadenoma
Illouz (2006)	(60)	Cushing's disease	3	Cabergoline	Tumor volume stabilization in one case

This study confirms the previous reports, showing that cabergoline treatment caused remission of Nelson's syndrome (57) and normalization of PRL, ACTH, and cortisol levels in a macroprolactinoma associated with Cushing's disease (58), suggesting that the DA agonist may be useful in restraining hormone secretion by neoplastic corticotrope cells. In addition, a previous *in vitro* study demonstrated that bromocriptine inhibits murine ACTH-secreting pituitary adenoma (AtT-20) cell proliferation, likely inducing apoptosis (59), further supporting the therapeutic use of DA agonists in the management of Cushing's disease. And indeed, three cases of persistent Cushing's disease have been described, where treatment with cabergoline induced a decrease or normalization in hypercortisolism, with stabilization of tumor volume in one case (60). Besides the DA agonists, SRIH analogs have been studied as possible pharmacological agents to treat ACTH-secreting pituitary adenomas. It has been recently demonstrated that human corticotrope tumors express multiple SSTR subtypes, and that the multiligand SRIH analog SOM230 significantly suppresses cell proliferation, as well as ACTH secretion, in primary cultures of human corticotrope tumors. These data suggest that SOM230 may have a role in the medical therapy of corticotrope tumors (61).

Together with SRIH analogs and DA agonists, chimeric compounds interacting with SSTR2 or SSTR5 and DR2 have been explored for their effects on cell viability. In a recent study, these compounds were capable of reducing cell viability in most (but not all) NFA primary cultures, having an inhibitory effect similar to selective SSTR or DR agonists. However, bromocriptine was the most effective agonists in reducing NFA cell viability (62).

In conclusion, the antiproliferative effect of SRIH analogs, DA agonists and chimeras, both *in vitro* (Table 1) and *in vitro* (Table 2), depends on tissue SSTR and DR expression pattern, the different binding profiles of the various agonists and SSTR and DR effector coupling. Further studies are therefore needed to elucidate the mechanisms underlying such antiproliferative effects and to verify the possible clinical applications.

Acknowledgements

References

1 Brazeau P & Guillemin R. Somatostatin: newcomer from the hypothalamus. *New England Journal of Medicine* 1974 **290** 963–964.

2 Giustina G, Peracchi M, Reschini E, Panerai AE & Pinto M. Dose–response study of the inhibiting effect of somatostatin on growth hormone and insulin secretion in normal subjects and acromegalic patients. *Metabolism* 1975 **24** 807–815.

3 Hall R, Snow M, Scanlon M, Mora B & Gomez-Pan A. Pituitary effects of somatostatin. *Metabolism* 1978 **27** (Suppl 1) 1257–1262.

4 Culler MD, Taylor JE & Moreau JP. Somatostatin receptor subtypes: targeting functional and therapeutic specificity. *Annals of Endocrinology* 2002 **63** 2S5–2S12.

5 Ferjoux G, Bousquet C, Cordelier P, Benali N, Lopez F, Rochaix P, Buscail L & Susini C. Signal transduction of somatostatin receptors negatively controlling cell proliferation. *Journal of Physiology* 2000 **94** 205–210.

6 Buscail L, Esteve JP, Saint-Laurent N, Bertrand V, Reisine T, O'Carroll AM, Bell GI, Schally AV, Vaysse N & Susini C. Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms. *PNAS* 1995 **92** 1580–1584.

7 Sharma K, Patel YC & Srikant CB. Subtype-selective induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. *Molecular Endocrinology* 1996 **10** 1688–1696.

8 Koper JW, Hofland LJ, van Koetsveld PM, den Holder F & Lamberts SW. Desensitization and resensitization of rat pituitary tumor cells in long-term culture to the effects of the somatostatin analogue SMS 201-995 on cell growth and prolactin secretion. *Cancer Research* 1990 **50** 6238–6242.

9 Hofland LJ, van Koetsveld PM, Wouters N, Waaijers M, Reubi JC & Lamberts SW. Dissociation of antiproliferative and antihormonal effects of the somatostatin analog octreotide on 7315b pituitary tumor cells. *Endocrinology* 1992 **131** 571–577.

10 Panetta R & Patel YC. Expression of mRNA for all five human somatostatin receptors (hSSTR1-5) in pituitary tumors. *Life Sciences* 1995 **56** 333–342.

11 Thapar K, Kovacs KT, Stefaneanu L, Scheithauer BW, Horvath E, Lloyd RV, Li J & Laws ER Jr. Antiproliferative effect of the somatostatin analogue octreotide on growth hormone-producing pituitary tumors: results of a multicenter randomized trial. *Mayo Clinic Proceedings* 1997 **72** 893–900.

12 Losa M, Ciccarelli E, Mortini P, Barzaghi R, Gaia D, Faccani G, Papotti M, Mangili F, Terreni MR, Camanni F & Giovanelli M. Effects of octreotide treatment on the proliferation and apoptotic index of GH-secreting pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5194–5200.

13 Cap J, Cerman J, Nemecek S, Marekova M, Hana V & Frysak Z. The influence of treatment with somatostatin analogues on morphology, proliferative and apoptotic activity in GH-secreting pituitary adenomas. *Journal of Clinical Neurosciences* 2003 **10** 444–448.

14 Ezzat S, Horvath E, Harris AG & Kovacs K. Morphological effects of octreotide on growth hormone-producing pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 113–118.

15 Bevan JS, Webster J, Burke CW & Scanlon MF. Dopamine agonists and pituitary tumor shrinkage. *Endocrine Reviews* 1992 **13** 220–240.

16 Bevan JS. The antitumoral effects of somatostatin analog therapy in acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1856–1863.

17 Renner U, Mojto J, Lange M, Muller OA, von Werder K & Stalla GK. Effect of bromocriptine and SMS 201-995 on growth of human somatotrophic and non-functioning pituitary adenoma cells *in vitro*. *European Journal of Endocrinolology* 1994 **130** 80–91.

18 Danila DC, Haidar JN, Zhang X, Katznelson L, Culler MD & Klibanski A. Somatostatin receptor-specific analogs: effects on cell proliferation and growth hormone secretion in human somatotroph tumors. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2976–2981.

19 Dasgupta P, Singh AT & Mukherjee R. Antiproliferative and GH-inhibitory activity of chimeric peptides consisting of GHRP-6 and somatostatin. *Biochemical Biophysical Research Communications* 1999 **259** 379–384.

20 Florio T, Thellung S, Corsaro A, Bocca L, Arena S, Pattarozzi A, Villa V, Massa A, Diana F, Schettini D, Barbieri F, Ravetti JL, Spaziante R, Giusti M & Schettini G. Characterization of the intracellular mechanisms mediating somatostatin and lanreotide inhibition of DNA synthesis and growth hormone release from dispersed human GH-secreting pituitary adenoma cells *in vitro*. *Clinical Endocrinology* 2003 **59** 115–128.

21 Ferrante E, Pellegrini C, Bondioni S, Peverelli E, Locatelli M, Gelmini P, Luciani P, Peri A, Mantovani G, Bosari S, Beck-Peccoz P, Spada A & Lania A. Octreotide promotes apoptosis in human somatotroph tumor cells by activating somatostatin receptor type 2. *Endocrine-Related Cancer* 2006 **13** 955–962.

22 Luciani P, Gelmini S, Ferrante E, Lania A, Benvenuti S, Baglioni S, Mantovani G, Cellai I, Ammannati F, Spada A, Serio M & Peri A. Expression of the antiapoptotic gene seladin-1 and octreotide-induced apoptosis in growth hormone-secreting and nonfunctioning pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 6156–6161.

23 Theodoropoulou M, Zhang J, Laupheimer S, Paez-Pereda M, Erneux C, Florio T, Pagotto U & Stalla GK. Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. *Cancer Research* 2006 **66** 1576–1582.

24 Florio T, Thellung S, Arena S, Corsaro A, Spaziante R, Gussoni G, Acuto G, Giusti M, Giordano G & Schettini G. Somatostatin and its analog lanreotide inhibit the proliferation of dispersed human non-functioning pituitary adenoma cells *in vitro*. *European Journal of Endocrinology* 1999 **141** 396–408.

25 Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D & Bruns C. Opportunities in somatostatin research: biological, chemical and therapeutic aspects. *Nature Reviews. Drug Discovery* 2003 **2** 999–1017.

26 Zatelli MC, Piccin D, Tagliati F, Bottoni A, Luchin A & degli Uberti EC. SHP-1 restrains cell proliferation in human medullary thyroid carcinoma. *Endocrinology* 2005 **146** 2692–2698.

27 Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC & Patel YC. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. *Science* 2000 **288** 154–157.

28 Culler MD. Evolving concepts in the quest for advanced therapeutic analogues of somatostatin. *Digestive and Liver Disease* 2004 **36** (Suppl 1) S17–S25.

29 Zatelli MC, Piccin D, Tagliati F, Ambrosio MR, Margutti A, Padovani R, Scanarini M, Culler MD & degli Uberti EC. Somatostatin receptor subtype 1 selective activation in human growth hormone- and prolactin-secreting pituitary adenomas: effects on cell viability, growth hormone and prolactin secretion. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 2797–2802.

30 Matrone C, Pivonello R, Colao A, Cappabianca P, Cavallo LM, Del Basso De Caro ML, Taylor JE, Culler MD, Lombardi G, Di Renzo GF & Annunziato L. Expression and function of somatostatin receptor subtype 1 in human growth hormone secreting pituitary tumors deriving from patients partially responsive or resistant to long-term treatment with somatostatin analogs. *Neuroendocrinology* 2004 **79** 142–148.

31 Zatelli MC, Piccin D, Bottoni A, Ambrosio MR, Margutti A, Padovani R, Scanarini M, Taylor JE, Culler MD, Cavazzini L & degli Uberti EC. Evidence for differential effects of selective somatostatin receptor subtype agonists on alpha-subunit and chromogranin A secretion and on cell viability in human non-functioning pituitary adenomas *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 5181–5188.

32 Oppizzi G, Cozzi R, Dallabonzana D, Orlandi P, Benini Z, Petroncini M, Attanasio R, Milella M, Banfi G & Possa M. Scintigraphic imaging of pituitary adenomas: an *in vivo* evaluation of somatostatin receptors. *Journal of Endocrinological Investigation* 1998 **21** 512–519.

33 Andersen M, Bjerre P, Schroder HD, Edal A, Hoilund-Carlsen PF, Pedersen PH & Hagen C. *In vivo* secretory potential and the effect of combination therapy with octreotide and cabergoline in patients with clinically non-functioning pituitary adenomas. *Clinical Endocrinology* 2001 **54** 23–30.

34 Colao A, Di Sarno A, Marzullo P, Di Somma C, Cerbone G, Landi ML, Faggiano A, Merola B & Lombardi G. New medical approaches in pituitary adenomas. *Hormone Research* 2000 **53** (Suppl 3) 76–87.

35 Gasperi M, Petrini L, Pilosu R, Nardi M, Marcello A, Mastio F, Bartalena L & Martino E. Octreotide treatment does not affect the size of most non-functioning pituitary adenomas. *Journal of Endocrinological Investigation* 1993 **16** 541–543.

36 Wood WM, Sarapura VD, Dowding JM, Woodmansee WW, Haakinson DJ, Gordon DF & Ridgway EC. Early gene expression changes preceding thyroid hormone-induced involution of a thyrotrope tumor. *Endocrinology* 2002 **143** 347–359.

37 Woodmansee WW, Kerr JM, Tucker EA, Mitchell JR, Haakinson DJ, Gordon DF, Ridgway EC & Wood WM. The proliferative status of thyrotropes is dependent on modulation of specific cell cycle regulators by thyroid hormone. *Endocrinology* 2006 **147** 272–282.

38 Hubina E, Nanzer AM, Hanson MR, Ciccarelli E, Losa M, Gaia D, Papotti M, Terreni MR, Khalaf S, Jordan S, Czirjak S, Hanzely Z, Nagy GM, Goth MI, Grossman AB & Korbonits M. Somatostatin analogues stimulate p27 expression and inhibit the MAP kinase pathway in pituitary tumours. *European Journal of Endocrinology* 2006 **155** 371–379.

39 Barrie R, Woltering EA, Hajarizadeh H, Mueller C, Ure T & Fletcher WS. Inhibition of angiogenesis by somatostatin and somatostatin-like compounds is structurally dependent. *Journal of Surgical Research* 1993 **55** 446–450.

40 Cristina C, Diaz-Torga G, Baldi A, Gongora A, Rubinstein M, Low MJ & Becu-Villalobos D. Increased pituitary vascular endothelial growth factor-a in dopaminergic D2 receptor knock-out female mice. *Endocrinology* 2005 **146** 2952–2962.

41 Stefaneanu L, Kovacs K, Horvath E, Buchfelder M, Fahlbusch R & Lancranjan L. Dopamine D2 receptor gene expression in human adenohypophysial adenomas. *Endocrine* 2001 **14** 329–336.

42 Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK & Low MJ. Pituitary lactotroph hyperplasia and chronic hyperprolactine-mia in dopamine D2 receptor-deficient mice. *Neuron* 1997 **19** 103–113.

43 Saiardi A, Bozzi Y, Baik JH & Borrelli E. Antiproliferative role of dopamine: loss of D2 receptors causes hormonal dysfunction and pituitary hyperplasia. *Neuron* 1997 **19** 115–126.

44 Bosse R, Fumagalli F, Jaber M, Giros B, Gainetdinov RR, Wetsel WC, Missale C & Caron MG. Anterior pituitary hypoplasia and dwarfism in mice lacking the dopamine transporter. *Neuron* 1997 **19** 127–138.

45 Iaccarino C, Samad TK, Mathis C, Kercret H, Picetti R & Borrelli E. Control of lactotrope proliferation by dopamine: essential role of signalling through D2 receptors ERKs. *PNAS* 2002 **99** 14530–14535.

46 Sarkar DK, Chaturvedi K, Oomizu S, Boyadjieva NI & Chen CP. Dopamine, dopamine D2 receptor short isoform, transforming growth factor (TGF)-beta1, and TGF-beta type II receptor interact to inhibit the growth of pituitary lactotropes. *Endocrinology* 2005 **146** 4179–4188.

47 Enjalbert A & Bockaert J. Pharmacological characterization of the D2 dopamine receptor negatively coupled with adenylate cyclase in rat anterior pituitary. *Molecular Pharmacology* 1983 **23** 576–584.

48 Martinez de la Escalera G, Guthrie J & Weiner RI. Transient removal of dopamine potentiates the stimulation of prolactin release by TRH but not VIP: stimulation via Ca2+/protein kinase C pathway. *Neuroendocrinology* 1988 **47** 38–45.

49 Ben-Jonathan N & Hnasko R. Dopamine as a prolactin (PRL) inhibitor. *Endocrine Reviews* 2001 **22** 724–763.

50 Takeuchi Y, Miyamoto E & Fukunaga K. Activation of the rat dopamine D2 receptor promoter by mitogen-activated protein kinase and Ca2+/calmodulin-dependent protein kinase II pathways. *Journal of Neurochemistry* 2002 **83** 784–796.

51 Bevan JS & Burke CW. Non-functioning pituitary adenomas do not regress during bromocriptine therapy but possess membrane-bound dopamine receptors which bind bromocriptine. *Clinical Endocrinology* 1986 **25** 561–572.

52 Liuzzi A, Tassi V, Pirro MT, Zingrillo M, Ghiggi MR, Chiodini I, Oppizzi G & Barberis M. Nonfunctioning adenomas of the pituitary. *Metabolism* 1996 **45** 80–82.

53 Renner U, Arzberger T, Pagotto U, Leimgruber S, Uhl E, Muller A, Lange M, Weindl A & Stalla GK. Heterogeneous dopamine D2 receptor subtype messenger ribonucleic acid expression in clinically nonfunctioning pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1368–1375.

54 Caccavelli L, Feron F, Morange I, Rouer E, Benarous R, Dewailly D, Jaquet P, Kordon C & Enjalbert A. Decreased expression of the two D2 dopamine receptor isoforms in bromocriptine-resistant prolactinomas. *Neuroendocrinology* 1994 **60** 314–322.

55 An JJ, Cho SR, Jeong DW, Park KW, Ahn YS & Baik JH. Anti-proliferative effects and cell death mediated by two isoforms of dopamine D2 receptors in pituitary tumor cells. *Molecular and Cellular Endocrinology* 2003 **206** 49–62.

56 Pivonello R, Ferone D, de Herder WW, Kros JM, De Caro ML, Arvigo M, Annunziato L, Lombardi G, Colao A, Hofland LJ & Lamberts SW. Dopamine receptor expression and function in corticotroph pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2452–2462.

57 Pivonello R, Faggiano A, Di Salle F, Filippella M, Lombardi G & Colao A. Complete remission of Nelson's syndrome after 1-year treatment with cabergoline. *Journal of Endocrinological Investigation* 1999 **22** 860–865.

58 T'Sjoen G, Defeyter I, Van De Saffele J, Rubens R & Vandeweghe M. Macroprolactinoma associated with Cushing's disease, successfully treated with cabergoline. *Journal of Endocrinological Investigation* 2002 **25** 172–175.

59 Yin D, Kondo S, Takeuchi J & Morimura T. Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine. *FEBS Letters* 1994 **339** 73–75.

60 Illouz F, Dubois-Ginouves S, Laboureau S, Rohmer V & Rodien P. Use of cabergoline in persisting Cushing's disease. *Annals of Endocrinology* 2006 **67** 353–356.

61 Batista DL, Zhang X, Gejman R, Ansell PJ, Zhou Y, Johnson SA, Swearingen B, Hedley-Whyte ET, Stratakis CA & Klibanski A. The effects of SOM230 on cell proliferation and adrenocorticotropin secretion in human corticotroph pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 4482–4488.

62 Gruszka A, Kunert-Radek J, Radek A, Pisarek H, Taylor J, Dong JZ, Culler MD & Pawlikowski M. The effect of selective sst1, sst2, sst5 somatostatin receptors agonists, a somatostatin/dopamine (SST/DA) chimera and bromocriptine on the "clinically non-functioning" pituitary adenomas *in vitro*. *Life Sciences* 2006 **78** 689–693.