CLINICAL STUDY

A luteinizing hormone-, alpha-subunit- and prolactin-secreting pituitary adenoma responsive to somatostatin analogs: *in vivo* and *in vitro* studies

A Saveanu¹, I Morange-Ramos², G Gunz¹, H Dufour³, A Enjalbert¹ and P Jaquet¹

¹Interactions Cellulaires Neuroendocriniennes, UMR6544, Centre National de la Recherche Scientifique, Institut Fédératif Jean Roche, Faculté de Médecine Nord, Boulevard Pierre Dramard, Marseille, France, ²Service d'Endocrinologie and ³Service de Neurochirurgie, Centre Hospitalier de Marseille, Hôpital de la Timone, 264 Rue Saint-Pierre, Marseille, France

(Correspondence should be addressed to P Jaquet, ICNE-UMR6544-CNRS, Institut Fédératif Jean Roche, Faculté de Médecine Nord, Boulevard Pierre Dramard, 13916 Marseille Cedex 20, France; Email: jaquet.p@jean-roche.univ-mrs.fr)

Abstract

Objective: Evaluation of the efficiency of somatostatin analogues in the treatment of a mixed luteinizing hormone (LH)-, α -subunit-, prolactin (PRL)-secreting pituitary adenoma.

Design: A 30-year-old woman, with amenorrhoea-galactorrhoea, presented with a pituitary macroadenoma. The endocrine evaluation showed high plasma levels of PRL, LH, and α -subunit inhibited by 65%, 65% and 33% respectively under octreotide test (200 μ g, s.c.). Long-term treatment with slow release (SR) lanreotide (30 mg/10 days, i.m.) restored menstrual cycles and normalized PRL values. Due to persisting supranormal levels of LH and α -subunit, and to the absence of tumoral shrinkage, the adenoma was resected by the transsphenoidal route.

Methods: *In vitro* characterization of the somatostatin receptor subtypes (SSTR) expression and functionality. Real-time polymerase chain reaction was performed to quantify the expression of SSTR mRNAs and functionality of the SSTRs was assessed in cell culture studies with various concentrations of native somatostatin (SRIF-14) and of analogues preferential for SSTR2 or SSTR5.

Results: This adenoma presented with high levels of SSTR2, SSTR3 and SSTR5 mRNAs, as compared with a series of gonadotroph adenomas. In cell culture studies, PRL, LH and α -subunit were inhibited by 60%, 47% and 33% respectively by SRIF-14 at a concentration of 10 nmol/l. The SSTR2 (BIM-23197, lanreotide) and SSTR5 (BIM-23268) preferential analogues both produced a partial 21–38% inhibition of PRL, LH, and α -subunit release.

Discussion: In this plurihormonal-secreting adenoma, the high efficacy of somatostatin analogues to inhibit PRL, LH and α -subunit secretion *in vivo* may be explained by the unusually high level of expression and by the functionality of both SSTR2 and SSTR5 receptor subtypes.

European Journal of Endocrinology 145 35–41

Introduction

In addition to its known suppressive effects upon growth hormone (GH) and thyrotrophin (TSH) secretion, there is some evidence that somatostatin may regulate gonadotrophin release. Such an inhibitory effect is only partial as a 30% luteinizing hormone (LH) suppression by somatostatin was found in normal men (1). In gonadotroph pituitary tumours, somatostatin receptors were identified on the tumour cell membranes (2, 3). Indeed, such findings led to the study of the effect of long-term octreotide therapy in patients with pituitary tumours which released luteinizing (LH) and follicle-stimulating (FSH) hormones and/or free α -subunit. If octreotide was poorly effective upon tumour shrinkage, a significant reduction in serum

FSH concentrations could be found in two out of four patients under long-term octreotide administration (4). More recently, it was shown by cell culture studies that somatostatin and its analogue, lanreotide, inhibited the proliferation of dispersed human non-functioning pituitary cells (5), which, in their majority, do synthesize LH, FSH or the α subunit (6–8). Such data, even if limited, indicated a possible inhibitory effect of somatostatin or its analogues in some gonadotroph tumours.

We report, in a women presenting with a pituitary macroadenoma co-secreting LH, α -subunit and PRL, the efficacy of the somatostatin agonist lanreotide in the control of hormonal hypersecretion. Subsequently, the patient underwent a selective surgical removal of the tumour. In this tumour, the quantitative analysis

of the different somatostatin receptor subtypes (SSTRs) as well as the functional responses obtained in cell culture with somatostatin analogues preferential for SSTR2 or SSTR5, may explain why only some, among the gonadotroph adenomas, may be responsive to somatostatin agonists.

Patient and Methods

Case report

A 30-year-old woman presented with amenorrhea and galactorrhea of 6 months duration. Prior to these symptoms, she presented with regular menses but with couple infertility for 3 years. A pituitary magnetic resonance imaging (MRI) scan found an intrasellar macroadenoma (maximal diameter 19×21 mm). Visual fields assessed by Goldman perimetry were normal. The basal plasma PRL value was $60 \mu\text{g/l}$ associated with supranormal plasma values of LH (30 U/l) and of α -subunit (7.2 U/l), contrasting with normal FSH plasma levels (7 U/l). Such an unusual pattern of PRL, LH, and α -subunit hypersecretion was not associated with TSH or GH hypersecretion. Estradiol and free testosterone plasma values were respectively 26 ng/l and 37 pmol/l . Insulin-like growth factor-I (IGF-I) levels were normal ($173 \mu\text{g/l}$) as were the free triiodothyronine (6 pmol/l), free thyroxine (15 pmol/l) and the 24-h urinary free cortisol (249 nmol/24 h) values.

The pharmacological evaluation was performed after stimulation by thyrotrophin releasing hormone (TRH; $200 \mu\text{g}$, i.v.) or by gonadotrophin releasing hormone (GnRH; $100 \mu\text{g}$, i.v.). The plasma LH, FSH, α -subunit, and PRL values were measured before each test and every 30 min for 2 h after TRH or GnRH. Similarly, the effects of the somatostatin analogue, octreotide (Sandostatin, Novartis, Basel, Switzerland; $200 \mu\text{g}$, s.c.) and of the dopamine agonist, quinagolide (Norprolac, Novartis; $150 \mu\text{g}$, oral) were tested on the inhibition of plasma levels of LH, FSH, α -subunit and PRL. Each test was performed on separate days. Under GnRH acute testing, the FSH, LH, and α -subunit plasma levels rose by 266, 279 and 42% respectively. A 112, 73 and 71% rise of PRL, LH and α -subunit values was also observed under acute TRH testing, while FSH values were unchanged. The dopamine agonist, quinagolide, produced only a slight (26% and 17%) maximal inhibition of plasma PRL and α -subunit and did not modify the LH or FSH plasma levels (Fig. 1, FSH not shown). In contrast, 2 to 4 h after octreotide injection, the plasma levels of PRL and LH decreased by 65% and those of α -subunit by 33% (Fig. 1). Such results, showing an overall much better inhibition of hormonal hypersecretion by octreotide than by quinagolide, led to the proposal of long-term treatment with a somatostatin analogue in order to further assess the

Figure 1 Inhibitory effects of quinagolide (150 mg , oral) and of octreotide ($200 \mu\text{g}$, s.c.) in acute tests. Plasma values of LH ($\diamond$), α -subunit ($\bullet$) and PRL ($\square$) are expressed as a percentage of baseline values at time 0.

efficacy of such a drug upon the control of hormone hypersecretion and upon the tumoral mass.

Long-term treatment with somatostatin analogue

The patient received an intramuscular injection of SR-Lanreotide 30 mg (Ipsen, Paris, France) repeated every 10 days for 3 months. The plasma levels of PRL, LH and α -subunit were measured hourly during the 6 h following the first injection and similarly each month before the next injection. The tumour mass was evaluated by MRI performed after 1 and 3 months of treatment. As shown in Fig. 2, during the 6 h following

Figure 2 Hormonal inhibition (% basal values) for LH ($\diamond$), α -subunit ($\bullet$) and PRL ($\square$) after the first injection of Lanreotide SR 30 mg and before the last injection of Lanreotide SR 30 mg after three months of treatment.

the first SR-Lanreotide injection, the plasma values of LH and PRL decreased by 80 and 84% respectively, while the α -subunit levels were only reduced by 54%. At the third month of treatment, the mean PRL value was in the normal range ($15 \pm 2 \mu\text{g/l}$), in accordance with the resumption of ovulatory cycles 48 days after the start of the treatment. Meanwhile, the LH and α -subunit plasma levels ($10 \pm 1 \text{ U/l}$ and $3.3 \pm 0.4 \text{ U/l}$) remained above the normal range. The MRI examination, at 3 months, did not find any significant tumour shrinkage (-13% decrease of the maximal diameter). Subsequently, the patient was operated on by the transphenoidal approach. On day 8 post surgery, plasma PRL, LH, and α -subunit values were $5 \mu\text{g/l}$, 1.9 U/l and 0.4 U/l respectively, and remained in these normal ranges in accord with the persistence of ovulatory cycles and with the occurrence of pregnancy 5 months after surgery.

In vitro studies

Quantification of somatostatin receptor subtypes

Part of the tumour was used for RNA extraction and purification using the guanidium isothiocyanate/phenol method followed by deoxyribonuclease I treatment, as previously published (9). The 5' exonuclease (Taq Man; Perkin Elmer Biosystems, Courtaboeuf, France) assay, which allows a direct proportional readout for the progression of PCR reactions, was used to quantify the 5 SSTRs mRNAs. The details of these experimental procedures have been presented elsewhere (10). For quantitation of data, SSTR mRNA levels were, in the same reaction, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and expressed as picograms SSTR per picogram GAPDH. Such an SSTR mRNA quantification was performed on 30–60 mg tissues from the mixed PRL–LH– α -subunit adenoma as well as on tissue from another 8 gonadotroph non-secreting pituitary macroadenomas previously collected and stored in liquid nitrogen. In all these tumour fragments, RT-PCR with specific

hormonal probes (9) confirmed the presence of the transcripts for β -LH, β -FSH or α -subunit as well as the absence of pro-opiomelanocortin (POMC), and GH mRNA expression. These data, confirming the gonadotroph origin of such tumours, are summarized in Table 1. In patients G1 and G2, PRL hypersecretion was of tumoral origin (PRL mRNA found in the tumour), while for G4, G7 and G8 PRL hypersecretion was interpreted as resulting from stalk compression.

Cell culture studies A part of the tumour, obtained after surgery, was dissociated by mechanical and enzymatic methods. The 4×10^6 dissociated cells obtained were plated in 24-multiwell culture dishes (3×10^4 cells/well) coated with extracellular matrix from bovine endothelial corneal cells, as previously described (11). Hormone release was assessed after a 24-h incubation period on day 1. On day 4 of culture, the effects of various concentrations of quinagolide, native somatostatin (SRIF-14; Sigma Chemical Company, Saint-Quentin Fallavier, France), lanreotide and the previously shown (12) preferential analogues to SSTR2, BIM-23197, and SSTR5, BIM-23268 (Biomeasure Inc., Milford, MA, USA), on PRL, LH and α -subunit secretion were measured after an 8-h period of incubation with these drugs. Each concentration of the drugs was tested in 4 different culture wells.

Results

SSTRs mRNA quantification

The quantification of the 5 SSTR mRNAs in the patient's tumour (G1, Fig. 3A) showed high expression of 3 SSTR subtypes: SSTR2 mRNA (327 pg/pg GAPDH), SSTR3 mRNA (950 pg/pg GAPDH) and SSTR5 mRNA (1396 pg/pg GAPDH). When the same analysis was performed in a series of 8 gonadotroph adenomas, a constant pattern of SSTR2 and SSTR3 mRNA expression was observed. The mean SSTR3

Table 1 Clinical characterization and hormonal status *in vivo* and *in vitro* (mRNA) of G1 and of eight other gonadotroph adenomas (G2–G9).

No.	Age	Sex	Hormonal status <i>in vivo</i>				Hormonal status <i>in vitro</i> (mRNA)					
			LH (U/l)	FSH (U/l)	α SU (U/l)	PRL ($\mu\text{g/l}$)	LH	FSH	α SU	PRL	GH	POMC
G 1	31	F	22	6	7	60	+	–	+	+	–	–
G 2	41	M	4	3	0.6	38	+	+	+	+	–	–
G 3	44	M	6	5	0.6	11	+	+	+	–	–	–
G 4	72	F	7	3	0.9	78	+	+	+	–	–	–
G 5	69	F	12	18	1	24	+	+	+	–	–	–
G 6	76	M	<1	1.3	0.3	14	+	–	+	–	–	–
G 7	56	F	6	11	1.3	66	+	+	+	–	–	–
G 8	68	F	17	4	1.4	75	+	+	+	–	–	–
G 9	58	M	6	4	0.8	12	–	+	+	–	–	–

α SU, alpha subunit.

Figure 3 Quantitative RT-PCR expression of SSTR2 (open bars), SSTR3 (grey bars) and SSTR5 (hatched bars) mRNAs in G1 (A) and in eight other gonadotroph adenomas (G2-G9; B). Results are expressed as pg/pg GAPDH (mean $\pm$ S.E.M. of 3 runs).

subtype expression (1413 ± 334 pg/pg GAPDH) was 12 times higher than that of SSTR2 mRNA (112 ± 35 pg/pg GAPDH). In most of these gonadotroph tumours (Fig. 3B), the SSTR5 subtype was not found. Only one other tumour (G2), which also coexpressed PRL mRNA with LH, FSH and α -subunit mRNAs (Table 1), presented with a high level of SSTR5 mRNA expression. Our mixed gonadotrophin-PRL secreting adenoma (G1), as compared with the other gonadotroph tumours, presented with the highest expression of both SSTR2 and SSTR5 mRNAs.

Cell culture studies

The basal level of hormone release into the culture medium by 1×10^6 cells during 24 h (day 1) was 660 ng for PRL, 165 mUI for LH, and 100 mUI for α -subunit. FSH, TSH and GH were not detected in the culture medium. These data confirmed the multi-secreting features of such a tumour as observed *in vivo*.

Effects of SRIF-14 and somatostatin analogues In this series of experiments, the dose-response inhibition of LH, α -subunit and PRL release was examined with 10^{-12} to 10^{-8} mol/l concentrations of SRIF-14, lanreotide, BIM-23197 and BIM-23268. As shown in Fig. 4, SRIF-14 produced a $60 \pm 2\%$, $47 \pm 3\%$ and $33 \pm 2\%$ maximal suppression of PRL, LH and α -subunit respectively, at a concentration of 10 nmol/l. The half-inhibitory effects (EC_{50}) were achieved at 100 pmol/l for the three hormones. The SRIF-14-induced suppression of PRL and LH was greater than that of α -subunit. Such a partial suppressive effect upon α -subunit secretion had already been observed *in vivo*, following acute testing with octreotide. As compared with the results obtained in the presence of various concentrations of SRIF-14, the dose-response

Figure 4 Dose-response LH ($\diamond$), α -subunit ($\bullet$) and PRL ($\square$) suppression curves obtained with SRIF-14, lanreotide, BIM-23197 and BIM-23268 (10^{-12} – 10^{-8} mol/l) in cells cultured from G1 adenoma. Results (mean $\pm$ S.E.M.) are expressed as percentage hormone suppression versus controls (C=medium alone).

inhibition of PRL, LH and α -subunit was only partial in the presence of lanreotide, BIM-23197 and BIM-23268. These drugs produced, at nanomolar concentrations, a maximal LH, α -subunit and PRL suppression ranging from 21 to 38%. The EC_{50} varied from 30 to 100 pmol/l, according to each compound. These data indicated that both SSTR2- (Lanreotide, BIM-23197) and SSTR5 (BIM-23268)-preferential analogues were able to suppress the hormone release. Nevertheless these SSTR2- or SSTR5-preferential analogues produced only a partial hormonal suppression as compared with that achieved by SRIF-14.

Effects of the dopamine agonist, quinagolide
Quinagolide, at the concentrations usually effective in inhibiting PRL release in prolactinomas ($EC_{50} = 30 \text{ pmol/l}$ (10)), was ineffective in the inhibition of α -subunit, LH and PRL release. Only at high quinagolide concentrations (1 to 10 nmol/l) was a 17–30% suppression of α -subunit, LH and PRL achieved (data not shown). Such results confirmed the poor sensitivity to the dopamine agonist in this tumour, as already observed *in vivo*.

Discussion

This report presents an unusual case of a multi-hormonal gonado-lactotroph secreting adenoma resistant to dopamine agonist and sensitive to somatostatin analogues in terms of inhibition of hormonal release. At least two individual cases of pituitary adenomas also cosecreting PRL and gonadotrophins have been reported in the literature (13, 14). One of these cases (14) presented as precocious puberty in a seven-year-old boy with high plasma levels of LH, FSH and PRL, which were not modified by octreotide. Another patient with an LH+ α -subunit secreting adenoma (15) showed a 57 to 70% inhibition of high plasma levels of LH and α -subunit under octreotide treatment. This patient, as in our observation, was resistant to acute and long-term bromocriptine administration. Our observation differs from most of the gonadotroph adenomas, characterized by immunohistochemical methods, which behave, *in vivo*, as non-functioning pituitary adenomas in the majority of cases. In fact, cell culture studies of gonadotroph adenomas have found, in most of the cases, the secretion of small amounts of FSH, LH and/or α -subunit (6–8). In these cell culture studies, an associated gonadotrophin and PRL co-secretion has also been reported in 8/99 cases (7).

The overall efficacy of somatostatin and its analogues is very partial when evaluated in gonadotroph adenomas. In three previous series, 29 gonadotroph adenomas were studied in cell culture (4, 16, 17). Somatostatin produced, *in vitro*, a partial (28–34%) inhibition of FSH, LH or α -subunit in less than 28% of these tumours. Such a score parallels the *in vivo* observations of a partial suppressive effect of octreotide upon gonadotrophin plasma levels in such patients. In one study concerning patients with α -subunit adenomas, a 27–35% reduction in α -subunit plasma levels was achieved in 2/6 patients treated with octreotide s.c. (200 $\mu\text{g/day}$) for 2 months (18). Increasing daily doses of octreotide up to 1200 $\mu\text{g/day}$ suppressed FSH plasma levels by only 7–17% in 2/4 patients with gonadotroph adenomas (4). The high suppressive effect of octreotide or lanreotide in our observation can be discussed in the light of our quantitative analysis of the somatostatin receptors in the tumour. In previous qualitative analyses, the non-functioning and gonadotroph adenomas were found to express mainly SSTR2

(63%) and SSTR3 (69%) mRNAs (5, 19–22). This is confirmed in all the tumours from our series, which constantly co-expressed SSTR2 and SSTR3 mRNA. The much higher relative expression of SSTR3 mRNA has already been stressed (22), but its functional significance is not yet explained. In our series, the level of SSTR2 mRNA expression was highly variable according to the tumours. The highest SSTR2 mRNA expression was found in tumour G1 and can explain the efficacy of octreotide and lanreotide in our observation, while both analogues are preferential mainly for SSTR2. In this adenoma, the high level of SSTR5 mRNA is also an unusual feature. The SSTR5 subtype has been found in only 10/35 of the non-functioning pituitary adenomas in previous studies (5, 22). In our mixed tumour, its presence is not surprising, while SSTR5 has been found highly expressed in PRL-secreting adenomas (10, 12). Such a SSTR5 receptor is functional while, in our culture study, BIM-23268, a highly preferential agonist for SSTR5, was able to produce a dose-dependent inhibition of PRL, as well as of LH and of α -subunit secretion. Interestingly, the native peptide, SRIF-14, achieved, in our case, significantly higher hormone suppression than that obtained with analogues highly preferential for SSTR5 or SSTR2. These data suggest cooperation between these two receptor subtypes, possibly by heterodimerization, as has been demonstrated using cloned human receptors (23), and already suggested in some GH-secreting pituitary adenomas (24).

If, from the data in the literature, a subset of gonadotroph adenomas is partially sensitive to somatostatin analogues in terms of hormone inhibition, the octreotide efficacy upon tumour shrinkage is less clear. Among 48 patients with non-functioning adenomas and gonadotrophinomas (25, 26), a significant (>20%) tumour shrinkage was documented in only 7 cases (15%). The SSTR scintigraphy using In111-pentetreotide was of poor predictive value in one study (25). A possible explanation of this latter finding is that In111-pentetreotide presents with binding affinities towards the SSTR subtypes different from those of octreotide. In particular, the binding affinity ratio SSTR3/SSTR2 was markedly higher for In111-pentetreotide than for octreotide (27).

In conclusion, even if unusual, some gonadotroph tumours can be sensitive to the somatostatin analogues, which inhibit the plasma levels of LH or α -subunit, as illustrated in our observation. Indeed, in the first instance, surgery remains the main therapeutic option. Nevertheless, in such macroadenomas, surgery is often partial and a subsequent therapeutic option has to be proposed in the presence of tumour remnants. In such a situation, the hormonal suppressive effects of somatostatin (or of dopamine) analogues can be checked. If such tumours prove sensitive to such drugs (28), long-term treatment could be proposed for

the suppression of hormonal release and could eventually prevent further tumoral growth.

Acknowledgements

The authors are grateful to Dr L'H Ouafik for using a TaqMan technology for real time quantitative PCR in the Assistance Publique Hopitaux de Marseille laboratory, and to F Fina, N Peralez and Dr D Morando for technical assistance. Dr M Culler (Biomeasure Inc., Milford, MA, USA) kindly provided the somatostatin analogues, BIM-23197 and BIM-23268. Alexandru Saveanu is funded by a research fellowship from the Conseil Regional Provence Alpes Cote d'Azur.

References

- Samuels MH, Henry P & Ridgway EC. Effects of dopamine and somatostatin on pulsatile pituitary glycoprotein secretion. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 217–222.
- Ikuyama S, Nawata H, Kato K, Karashima T, Ibayashi H & Nakagaki H. Specific somatostatin receptors on human pituitary adenoma cell membranes. *Journal of Clinical Endocrinology and Metabolism* 1985 **61** 666–671.
- Reubi JC, Heitz PU & Landolt AM. Visualization of somatostatin receptors and correlation with immunoreactive growth hormone and prolactin in human pituitary adenomas: evidence for different tumor subclasses. *Journal of Clinical Endocrinology and Metabolism* 1987 **65** 65–73.
- De Bruin TWA, Kwekkeboom DJ, Van't Verlaet JW, Reubi JC, Krenning EP & Lamberts SWJ. Clinically non-functioning pituitary adenoma and octreotide response to long-term treatment and studies *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 1310–1317.
- Florio T, Thellung S, Arena S, Corsaro A, Spaziante R, Gussoni G *et al*. 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.
- Asa SL, Gerrie BM, Singer W, Horvath E, Kovacs K & Smith HS. Gonadotropin secretion *in vitro* by human pituitary null cell adenomas and oncocytomas. *Journal of Clinical Endocrinology and Metabolism* 1986 **62** 1011–1119.
- Asa SL, Cheng Z, Ramyar L, Singer W, Kovacs K, Smith HS *et al*. Human pituitary null cell adenomas and oncocytomas *in vitro*: effects of adenylylating hormones and gonadal steroids on hormone secretion and tumor cell morphology. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 1128–1134.
- Katznelson L, Alexander JM, Bikkal HA, Kliman B, Jameson JL, Hsu DW *et al*. Imbalanced follicle-stimulating hormone beta-subunit hormone biosynthesis in human pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 1343–1351.
- Pellegrini-Bouiller I, Manrique C, Gunz G, Grino M, Zamora AJ, Figarella-Branger D *et al*. Expression of the members of the Ptx family of transcription factors in human pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2212–2220.
- Jaquet P, Ouafik L, Saveanu A, Gunz G, Fina F, Dufour H *et al*. Quantitative and functional expression of somatostatin-receptor subtypes in human prolactinomas. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3268–3276.
- Jaquet P, Gunz G & Grisoli F. Hormonal regulation of prolactin release by human prolactinoma cells cultured in serum-free conditions. *Hormone Research* 1985 **22** 153–163.
- Shimon I, Taylor JE, Dong JZ, Bitonte RA, Kim S, Morgan B *et al*. Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation. *Journal of Clinical Investigation* 1997 **4** 789–798.
- Faggiano M, Criscuolo T, Perrone L, Quarto C & Sinisi AA. Sexual precocity in a boy due to hypersecretion of LH and prolactin by a pituitary adenoma. *Acta Endocrinologica* 1983 **102** 167–172.
- Ambrosi B, Bassetti M, Ferrario R, Medri G, Giannattasio G & Faglia G. Precocious puberty in a boy with a PRL-, LH- and FSH-secreting pituitary tumour: biosynthetic characterization and clinical studies. *Acta Endocrinologica* 1990 **122** 569–576.
- Vos P, Croughs RJ, Thijssen JH, Van't Verlaet JW & Van Ginkel LA. Response of luteinizing hormone secreting pituitary adenoma to a long-acting somatostatin analogue. *Acta Endocrinologica* 1988 **118** 587–590.
- Klibanski A, Alexander JM, Bikkal HA, Hsu DW, Swearingen B & Zervas NT. Somatostatin regulation of glycoprotein hormone and free subunit secretion in clinically nonfunctioning and somatotroph adenomas *in vitro*. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1248–1255.
- Hofland LJ, De Herder WW, Visser-Wisselaar HA, Van Huffelen C, Waaijers M, Zuyderwijk J *et al*. 1997 Dissociation between the effects of somatostatin (SS) and octapeptide SS-analogs on hormone release in a small subgroup of pituitary and islet cell tumors. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 3011–3018.
- Katznelson L, Oppenheim DS, Coughlin JF, Kliman B, Schoenfeld DA & Klibanski A. Chronic somatostatin analog administration in patients with α -subunit-secreting pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 1318–1325.
- Greenman Y & Melmed S. Heterogeneous expression of two somatostatin receptor subtypes in pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 398–403.
- Greenman Y & Melmed S. Expression of three somatostatin receptor subtypes in pituitary adenomas: evidence for preferential SSTR5 expression in the mammosomatotroph lineage. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 724–729.
- Schaer JC, Waser B, Mengod G & Reubi JC. Somatostatin receptor subtypes sst₁, sst₂, sst₃ and sst₅ expression in human pituitary, gastroentero-pancreatic and mammary tumors: comparison of mRNA analysis with receptor autoradiography. *International Journal of Cancer* 1997 **70** 530–537.
- Nielsen S, Møllemaer S, Rasmussen LM, Ledet T, Astrup J, Weeke J *et al*. Gene transcription of receptors for growth hormone-releasing peptide and somatostatin in human pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2997–3000.
- Rocheville M, Lange DC, Kumar U, Sasi R, Patel RC & Patel YC. Subtypes of the somatostatin receptor assemble as functional homo- and heterodimers. *Journal of Biological Chemistry* 2000 **275** 7862–7869.
- Saveanu A, Gunz G, Dufour H, Caron P, Fina F, Ouafik L *et al*. BIM-23244, a somatostatin receptor subtype 2- and 5-selective analog with enhanced efficacy in suppressing growth hormone (GH) from octreotide-resistant human GH-secreting adenomas. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 140–145.
- Plockinger U, Bader M, Hopfenmüller W, Saeger W & Quabbe HJ. Results of somatostatin receptor scintigraphy do not predict pituitary tumor volume- and hormone-response to octreotide therapy and do not correlate with tumor histology. *European Journal of Endocrinology* 1997 **136** 369–376.
- Warnet A, Harris AG, Renard E, Martin D, James-Deidier A & Chaumet-Riffaud P. French Multicenter Octreotide Study Group. A prospective trial of octreotide in 24 patients with visual defects caused by non-functioning and gonadotropin-secreting pituitary adenomas. *Neurosurgery* 1997 **41** 786–797.
- Reubi JC, Schaer JC, Waser B, Wenger S, Heppeler A, Schmitt JS *et al*. Affinity profiles for human somatostatin receptor subtypes

SST1-SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use. *European Journal of Nuclear Medicine* 2000 **27** 273–282.

- 28 Andersen M, Bjerre P, Schroder HD, Edal A, Hoilund-Carlsen PF, Pedersen PH *et al.* *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.

Received 10 January 2001

Accepted 19 March 2001