Partial tachyphylaxis to somatostatin (SST) analogues in a patient with acromegaly: the role of SST receptor desensitisation and circulating antibodies to SST analogues

Shahid T Wahid, Peter Marbach, Barbara Stolz, Margaret Miller, Robert Andrew James and Steve G Ball
Endocrine Unit, Royal Victoria Infirmary, Newcastle Hospitals NHS Trust and University of Newcastle, Newcastle upon Tyne, NE1 4LP, UK and Novartis Pharma, Basle, Switzerland

(Correspondence should be addressed to S G Ball, School of Clinical Medical Sciences, The Medical School, 4th Floor William Leech Building, University of Newcastle upon Tyne, NE2 4HH, UK; Email: S.G.BALL@newcastle.ac.uk)

Abstract

Objective: Somatostatin (SST) analogues are a key option in the management of a variety of conditions, including acromegaly. Tachyphylaxis to SST analogues is not documented in acromegaly. We describe such a phenomenon.

Design and methods: A 74-year-old female with acromegaly previously treated with 90Y implant, external radiotherapy and thrice daily s.c. octreotide had stable GH levels of 19 mU/l. GH progressively rose following switches to lanreotide and depot octreotide as Sandostatin LAR: from 29 to 126 mU/l. Magnetic resonance imaging and 111In-pentetreotide scanning revealed no tumour growth or alteration in SST receptor (SSTR) status. Tachyphylaxis to SST analogues was considered. Therapy was discontinued and re-introduced in daily 200 mg/24 h increments by continuous s.c. infusion, to a maximum of 1000 mg/24 h, and maintained over 3 weeks with daily, followed by weekly, GH profiles. Competitive 125I-octreotide radioligand binding assays measured in vitro bio-activity of anti-SST analogue antibodies. In vitro SSTR binding studies utilised SSTR-expressing rat cortex membrane.

Results: Median GH fell by 93% from 504 to 39.5 mU/l and rose reproducibly on continued infusion to 120 mU/l. Octreotide withdrawal for 16 h produced a 64% increase in sensitivity. High-affinity IgG anti-lanreotide IC50 = 187 pmol/l and anti-octreotide IC50 = 82 nmol/l antibody, with no cross-reactivity with natural SST, was demonstrated. In vitro inhibition of 125I-octreotide SSTR binding by anti-SST analogue crossreacting antibody was observed at 1:1 serum dilution.

Conclusions: This is the first report of tachyphylaxis to SST analogues in acromegaly. We believe that the short time course of resensitisation following acute octreotide withdrawal is suggestive of an effect(s) on receptor function or on the receptor signal transduction cascade at sites further downstream, rather than an immune-mediated phenomenon.

Introduction

Acromegaly is a rare condition, with an incidence of 2.8–4.0 per million per year (1–3). The mortality rate in acromegalic patients is twice that of the general population (4). This increased mortality rate can be reduced to the level of the general population by the maintenance of a growth hormone (GH) level less than 5 mU/l (5–7). Surgical therapy remains the first-line option in the treatment of acromegaly. With the emergence of somatostatin (SST) analogues in the form of octreotide (Sandostatin, Novartis) (8, 9), and its depot preparation Sandostatin LAR (Novartis) (10, 11), and in the form of lanreotide, and its depot preparation Somatuline LA (Ipsen) (12), medical therapy has become an attractive option for a number of patients, as both adjunctive and potential first-line therapy. SST analogues are used in the management of a variety of other conditions (13–15). Although the development of resistance to SST analogues is recognised in the treatment of neuroendocrine tumours (16), extensive trial evidence (8–12) has not documented the development of tolerance to SST analogue therapy in acromegaly. The mechanism of this differential response is not clear, although it may reflect the differential expression of SST receptor (SSTR) subtypes on neuroendocrine tumours.

We describe a case of partial tachyphylaxis to SST analogue therapy in a patient with long-standing acromegaly, and present in vivo and in vitro data demonstrating the mechanism(s) that may underlie this phenomenon.
Patient and methods

Patient

The patient, a 74-year-old female, had a 23 year history of acromegaly secondary to a pituitary tumour co-secreting GH and thyrotrophin (TSH). End-organ complications included type 2 diabetes mellitus, ischaemic heart disease, hypertension, and widespread osteoarthritis. Past medical history included a right nephrectomy for a staghorn calculus, radioactive iodine therapy for thyrotoxicosis, and bilateral carpal tunnel syndrome requiring treatment with surgery on two occasions. Initial treatment for acromegaly had been with a 90Y intrasella implant (150 000 Gy). Pituitary biopsy at the time of the implant revealed a poorly granulated acidophilic adenoma. Further treatment with external beam pituitary radiotherapy was given 14 years after presentation because of persistent disease. She was subsequently shown to be SST analogue sensitive; following a 50 μg test dose of s.c. octreotide GH fell from a mean of 24 mU/l to a nadir of 1.9 mU/l over 2 h. The patient received octreotide treatment in the form of three s.c. injections a day for a total of 7 mg t.d.s. Despite this, her acromegaly remained active, with an insulin-like growth factor (IGF-I) level of 52 ng/l (normal 9 – 49 ng/l) and a mean GH of 19 mU/l.

Because of deteriorating osteoarthritis leading to difficulties with self-administration of s.c. injections, the patient was switched to long-acting i.m. SST analogue therapy when it became available. Initially, this was with weekly Somatuline LA. A progressive rise in mean GH to a maximum of 76 mU/l and worsening joint pain were noted. A further switch to Sandostatin LAR resulted in an initial improvement in GH levels, followed by a continued rise over 3 months from a nadir of 29 mU/l to a peak of 126 mU/l, despite escalating dose titration (Table 1). As before, this was clinically mirrored by deterioration in symptoms of joint pain. The development of tachyphylaxis to SST analogues was considered.

Methods

Imaging Tumour size was assessed by serial magnetic resonance imaging (MRI). Tumour expression of SSTRs was determined by 111In-pentetreotide scanning, with pituitary images at 12 and 24 h after i.v. injection of 110 MBq 111In-pentetreotide (Mallinckrodt, Netherlands). Twenty-four hour planar images were constructed using a Camstar XR/T gamma camera at gamma emissions of 171 and 245 KeV with a 20% window on each.

Response of GH and TSH to incremental doses of octreotide: demonstration of desensitisation To test for the occurrence of progressive desensitisation to octreotide, treatment (Sandostatin LAR 40 mg) was discontinued for a washout period of 4 weeks, then re-introduced as a continuous s.c. infusion (c.s.i.). The initial dose was 200 μg/24 h, increasing by daily increments of 200 μg to a final dose of 1000 μg/24 h. The final dose of 1000 μg/24 h was continued for 3 weeks. Blood sampling for GH, TSH and octreotide levels was performed 24 h after initiation of a given octreotide dose and immediately prior to dose or pump change. Samples were collected every hour for 4 h. Median GH and TSH values, with a 25 – 75% interquartile (IQ) range, were calculated for each series. The study protocol was repeated on two separate occasions.

Response of GH to re-introduction of octreotide after short-term withdrawal To test if short-term withdrawal of octreotide was associated with subsequent resensitisation to SST analogues, octreotide was discontinued for 16 h during week 2 of the 1000 μg/24 h phase of the study. Treatment was reintroduced at the same dose. Samples for GH levels were taken immediately prior to stopping treatment and again 24 h after re-introduction. As before, samples were collected hourly for 4 h.

Detection and characterisation of anti-SST analogue antibodies in patient serum

Anti-octreotide antibody titre determination. Serum anti-octreotide antibody titre was determined by a radio metric assay using a 125I-Tyr3-octreotide tracer. One hundred microlitres of patient serum diluted with pooled human serum (serial dilution from 1:0 to 1:100 000) were incubated with 100 μl tracer (total counts/tube ~18 000 c.p.m.) at 4 °C for 24 h. Specific counts representing bound tracer were measured after charcoal separation. Antibody titre was defined as the serum dilution at which there was at 50% inhibition of tracer binding, i.e. IC50.

RIA for testing specificity of human anti-octreotide antibody. Anti-octreotide antibody-positive human serum was used as the antiserum to set up a RIA for

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<th>Time (week)</th>
<th>Sandostatin LAR (mg/month)</th>
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<tr>
<td>4</td>
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testing specificity of the antibody. In a competitive RIA, the binding of $^{125}$I-Tyr$^1$-octreotide (tracer) was challenged by octreotide, lanreotide and SST-14. One hundred microlitres of the antibody-positive patient serum in 1:300 dilution were incubated with 100 μl of tracer and increasing concentrations of octreotide, lanreotide and SST-14. After an overnight incubation at 4°C, 500 μl of a charcoal suspension (charcoal 1%, plasma 1% in PBS) were added. The tubes were incubated for 10 min at 4°C and centrifuged for 15 min at 4000 r.p.m. at 4°C. One hundred microlitres of the supernatant were transferred into counting tubes, and counting performed in a gamma counter for 5 min.

**Anti-octreotide antibody-positive human antiserum characterisation.** Affinity characterisation (Scatchard analysis) was performed by a specific RIA measuring bound tracer concentrations at constant antiserum dilution (1:300 and 1:20 000 for patient serum and rabbit anti-octreotide used as a positive control respectively) and varying concentrations of $^{125}$I-Tyr$^1$-octreotide tracer (2.13 μCi/nmol). Standard rabbit anti-octreotide antiserum was used as a reference. To investigate antibody specificity with respect to crossreactivity with other SST analogues, binding of the tracer was challenged by octreotide, lanreotide and SST-14 in the same competitive RIA system. To characterise the subclass of immunoglobulin (Ig) responsible for anti-SST antibody activity, additional studies were performed using $^{125}$I-Tyr$^3$-octreotide bound to patient serum and specific anti-human Ig antibodies (anti-human IgA, IgG, IgE and IgM).

**Properties of anti-SST analogue antibodies: ability to displace $^{125}$I-Tyr$^1$-octreotide from SSTRs in vitro**

The ability of anti-SST analogue antibodies in patient serum to displace $^{125}$I-Tyr$^1$-octreotide from SSTR binding sites was tested using a radioligand binding assay as described previously (17). Briefly, octreotide and serial (1:1, 1:2, 1:5, 1:10 and 1:100) dilutions of patient serum were used as separate competitors of $^{125}$I-Tyr$^3$-octreotide binding to rat cortex membrane expressing SSTRs. Rat cortex membranes were diluted to 50 μg protein per assay tube in 10 mmol/l Hepes buffer (pH 7.6) containing 10 mmol/l MgCl$_2$ and 20 μg/ml bacitracin. Binding assays consisted of 70 μl radioligand, 30 μl buffer or increasing dilutions of patient serum and 200 μl cortex membrane suspension. Tubes were incubated for 60 min, at room temperature, with approximately 20 000 c.p.m. of $^{125}$I-Tyr$^3$-octreotide per tube. Incubation was stopped by rapid filtration through Whatman GF/C glass fibre filters, subsequently washed with 20 ml Tris buffer (10 mmol/l Tris–HCl, 150 mmol/l NaCl, pH 7.5, 4°C). Specific radioligand binding was defined as total binding minus binding in the presence of 10$^{-6}$ mol/l octreotide (non-specific binding). The experiment was carried out in triplicate.

**Hormone assays** GH was measured by an in-house IRMA assay using a polyclonal capture antibody and a second $^{125}$I-labelled anti-human GH monoclonal antibody. The interassay coefficient of variation (CV) was 4.7. TSH was measured by non-isotopic sandwich assay using a Bayer Immuno-1 Analyser, with an interassay CV of 4.9 (Bayer, Germany).

**Results**

**Progressive rise in GH does not reflect tumour progression or demonstrable loss of SSTR expression**

To determine whether the progressive rise in GH levels reflected tumour progression, pituitary MRI was repeated. Comparison with previous imaging demonstrated no change in tumour size (Fig. 1A and B). To determine the SSTR status of the tumour, and define whether the progressive rise in GH reflected alteration in SSTR responsiveness through alteration in tumour SSTR expression, we performed $^{111}$In-pentetreotide imaging. This demonstrated homogeneous uptake of $^{111}$In-pentetreotide throughout an enlarged pituitary, consistent with continued expression of SSTRs by the pituitary tumour (Fig. 2).

**Desensitisation is demonstrated during incremental increases in octreotide treatment**

Following the 4 week washout period the median plasma GH and TSH rose to 504 mU/l (IQ range 464–578) and 50 mU/l (IQ range 50–52) respectively. Introduction of octreotide, at a dose of 200 μg/24 h by c.s.i., produced a prompt fall in both plasma GH and TSH to 93 and 58% of baseline values respectively (Fig. 3). This response was maintained up to a dose of 800 μg/24 h c.s.i. Thereafter, both GH and TSH rose, consistent with some reduction in responsiveness to octreotide. The data do not demonstrate loss of responsiveness, peak GH and TSH levels being some 20% of post-washout values. The rises in GH and TSH were mirrored by clinical deterioration in symptoms of joint pain. Similar data were obtained when the protocol was repeated (data not shown).

**Resensitisation to octreotide is demonstrated on re-introduction of treatment after acute short-term withdrawal**

To determine whether resensitisation to octreotide could be demonstrated following acute withdrawal, octreotide treatment (1000 μg/24 h by c.s.i.) was stopped for 16 h. Twenty-four hours after re-introduction of octreotide 1000 μg/24 h by c.s.i., plasma GH levels were 64% lower than prior to drug withdrawal (Fig. 4). These data are consistent with resensitisation to octreotide after short-term withdrawal.

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**Characterisation of anti-SST analogue antibodies**

*In vitro* studies demonstrated the presence of a high titre (1:318) anti-octreotide antibody in the patient’s serum. This represents one of the highest anti-octreotide antibody titres obtained so far in our hands. Double antibody studies demonstrated the anti-octreotide antibody to be an IgG antibody (data not shown).

Figure 5 shows the Scatchard plot demonstrating the affinity of the patient’s serum for octreotide compared with that of a specific rabbit anti-octreotide antiserum. A biphasic plot was obtained for the patient serum, indicating a heterogeneous population of antibodies. The calculated affinity constant of the high-affinity antibody population of the patient sample (representing ~50% of the total amount) was comparable to that of the specific rabbit anti-octreotide serum ($K_a = 3.9 \times 10^{10}$ l/mol and $3.1 \times 10^{10}$ l/mol respectively), confirming the presence of a high-affinity antibody.

As one can see in Fig. 6, antibody(ies) in the patient serum has a higher affinity for lanreotide than for octreotide.
octreotide, with an IC$_{50}$ for lanreotide and octreotide of 187 pmol/l and 82 nmol/l respectively. Therefore, one might conclude that the antibody was primarily developed to lanreotide rather than to octreotide. Interestingly, competitive binding data indicate no significant binding of SST-14 by the patient serum. Anti-SST analogue antibodies precluded the accurate measurement of circulating octreotide.

The role of anti-SST analogue antibodies in decreased responsiveness to octreotide: in vitro displacement of octreotide from SSTRs by patient serum

The ability of anti-SST analogue antibodies contained in the patient’s serum to interfere with octreotide binding to SSTRs was tested by competitive binding assay in vitro. Tolerance to SST analogues
Small displacement of $^{125}\text{I}-\text{Tyr}^3$-octreotide by control to the patient’s serum, being much greater than the dose-dependent manner. This displacement was specific tide from rat cortex membrane expressing SSTRs in a human serum in which anti-SST analogue antibodies had not been demonstrated.

Discussion

We have documented a case of acromegaly in which the institution of long-acting SST analogue therapy was associated with a progressive rise in GH levels, not apparent on previous treatment with intermittent s.c. injections of octreotide. Imaging data excluded tumour progression as a cause of the progression. Additional nuclear medicine studies demonstrated that tumour SSTR expression was also maintained, although we were unable to quantify absolute SSTR expression or relate the data to SSTR expression prior to development of tachyphylaxis. Further clinical studies demonstrated both desensitisation to SST analogue therapy during incremental increases in treatment and resensitisation on re-introduction of treatment after short-term withdrawal. We believe these data are consistent with the development of partial tachyphylaxis to SST analogues.

Desensitisation to octreotide has been documented in both pituitary tumour (18, 19) and other tumour (20) cell lines in vitro, and in an in vivo mouse model (20). There is no previous report of tachyphylaxis to SST analogue therapy in patients with acromegaly. However, acquired resistance to the effects of octreotide has been documented in the treatment of gut-hormone secreting and pancreatic tumours (16, 21–23). In two cases of insulinoma and vipoma described by Lamberts et al. (23), s.c. octreotide therapy resulted in an initial biochemical and clinical improvement, followed by progressive biochemical and clinical relapse after 7 and 14 days of therapy respectively. Potential mechanisms of the acquired resistance were not explored. The fact that resensitisation was not observed in the short interval between dose increments suggests a different underlying mechanism from that in the case we describe. Given these data, the absence of tachyphylaxis to SST analogues in somatotrophinomas is a paradox. It may reflect differential SSTR subtype expression, some subtypes being more prone to the phenomenon than others. However, alternative mechanisms may be involved.

Before thorough investigation, two broad potential mechanisms for the partial tachyphylaxis to SST analogues were postulated: interference in analogue action by SSTR analogue antibodies; and alteration in skin reaction at the injection site and treatment resistance. The documented incidence of antibody production to octreotide was thought to be low (24). Kaal et al. (25) have recently reported a 77–81% incidence of antibodies to octreotide administered intranasally after a mean exposure of 9 months, a 27% incidence for octreotide administered s.c. after a mean exposure of 3 years and no occurrence of antibodies to i.m. Sandostatin LAR after a mean exposure of 2.5 years. Furthermore, the antibodies did not alter the GH or IGF-I status of any patients. There are few data on the impact of antibody formation on the clinical efficacy of SST analogues (26–28). To our knowledge, there are two published studies specifically looking at the impact of anti-SST analogue antibody formation on the response of patients with acromegaly. In the case presented by Kendall-Taylor et al. (26) a low anti-octreotide antibody titre was associated with a skin reaction at the injection site and treatment resistance. However, evidence linking the antibody to the resistance was lacking. Moreover, the poor initial fall in mean GH with octreotide treatment (28.7%) in

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Figure 7 Displacement of $^{125}\text{I}-\text{Tyr}^3$-octreotide from rat cortex membrane binding sites by patient plasma. $^{125}\text{I}-\text{Tyr}^3$-Octreotide was incubated with rat cortex membrane SSTRs in the presence of serial dilutions of patient plasma containing anti-SST antibodies. Patient plasma displaced octreotide from rat cortex membrane binding sites in a dose-dependent manner. Binding of $^{125}\text{I}-\text{Tyr}^3$-octreotide in the presence of patient serum was compared with that in the presence of a 1:1 dilution of human plasma not containing anti-SST analogue antibodies (negative control plasma). Non-specific binding was defined as $^{125}\text{I}-\text{Tyr}^3$-octreotide bound to cortex membrane in the presence of $10^{-6}\text{ mol/l octreotide. Individual experiments were performed in triplicate.}$ Data represent means, $n = 3. Statistical analysis (patient plasma vs negative control) was by ANOVA.
that case report strongly suggests the patient was a poor responder to SST analogues and that the anti-octreotide antibody may have played a minor contributory role. This is in contrast to the case we describe, in which there was a 93% initial response to octreotide treatment. It is of interest to note that there was no skin reaction at the sites of SST analogue injection in our patient. In the two cases presented by Orskov et al. (27), anti-octreotide antibody was specifically measured, and was IgG in nature. In both patients the IgG antibody prolonged the half-life of octreotide, resulting in greater GH suppression. In the case presented by Kwekkeboom et al. (28) anti-octreotide antibodies were visualised using 111In-octreotide scintigraphy. Increased accumulation of 111In-octreotide was noted at injection sites, and was confirmed to be secondary to anti-octreotide antibodies on histological examination. The imaging lent circumstantial support to both a prolonged circulation time and reduced effectiveness of octreotide coupled to antibody.

Having been stable on s.c. octreotide our patient was converted to Sandostatin LA and subsequently Sandostatin LAR. Given this time course, it is interesting to note that the patient’s serum had a higher affinity for lanreotide than for octreotide (Fig. 6). This raises the possibility that the primary immune reaction was to Sandostatin LA rather than octreotide.

Although we have demonstrated the potential of anti-SST analogue antibody to interfere with SST analogue action in vitro, we are reluctant to propose that the antibody we have characterised is the cause of the tachyphylaxis we have observed. In vitro activity may not reflect behaviour in vivo. In addition, we feel that the time course of desensitisation following acute octreotide withdrawal (Fig. 4) is not consistent with an immune-mediated phenomenon, and is more suggestive of a receptor or post-receptor mechanism. This may reflect altered tumour properties resulting from selective clonal expansion over time, e.g. the development and growth of a clone with properties different from that of the original tumour. This may be a random event, or reflect the influence of previous treatment.

Desensitisation at a receptor or post-receptor level could reflect a reduction in the total number of SSTRs, a selective reduction in the expression of specific SSTR subtypes, or a post-SSTR event interfering with receptor–effector coupling. 111In-pentetreotide imaging in this patient supports the maintenance of SST expression despite concurrent desensitisation in this case. There are five subtypes of SSTR (SSTR1–5), each a member of the super-family of G protein-coupled receptors (29). SSTR activation can influence a number of intracellular signal cascades through inhibition of adenyl cyclase; activation of tyrosine phosphatases; activation of phospholipase C; activation of mitogen-activated protein kinase; and alteration in cell membrane conductance (30). Each component of the receptor–effector coupling pathway could be potentially involved in SSTR desensitisation. Desensitisation in the continued presence of agonist is a feature of SSTR-mediated signalling (31). This has been attributed to SSTR down-regulation (19, 32) or alterations in receptor–effector coupling brought about through specific regions of the cytoplasmic tail of the SSTR (33). However, the responses of SSTR2 and SSTR5 to continuous agonist stimulation may differ from that of the other SSTRs. Continuous administration of both native SST (34) and SST analogues (20) results in SSTR2 up-regulation and continued efficacy; whilst a combination of recycling and recruitment of spare receptors protects SSTR5 against desensitisation (35). Such selective regulation may underlie the variable responses of different SST-responsive targets to long-term treatment observed in vivo. We feel the demonstration of short-term resensitisation, and maintenance of SSTR expression as determined by 111In-pentetreotide scintigraphy in this case favour a post-receptor mechanism as an explanation for the observed tachyphylaxis in this patient. We believe this to be the first reported case of partial tachyphylaxis to SST analogue therapy in acromegaly. Because of the increasing frequency and diversity of such therapy, further studies are warranted to elucidate the mechanism(s) of this phenomenon, which can result in reduced clinical efficacy.

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