Effects of somatostatin analogs on glucose homeostasis in rats

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Abstract

Pasireotide (SOM230) is a multireceptor-targeted somatostatin analog with high binding affinity for sstr1,2,3 and sstr5. The effects of pasireotide and octreotide on blood glucose, insulin, and glucagon levels in rats were evaluated alone and in combination. Single-dose s.c. pasireotide acutely elevated plasma glucose, whereas single-dose s.c. octreotide had no or a small hypoglycemic effect. Glucose elevation with s.c. pasireotide was transient with tachyphylaxis after repeated or continuous administration. Pasireotide and octreotide caused similar inhibitory effects on insulin secretion, whereas pasireotide had a weaker inhibitory effect on glucagon secretion than octreotide. Continuous infusion of pasireotide or injection of pasireotide long-acting release (LAR) resulted in only small and transient elevations of plasma glucose. Based on these results, and differences in the sstr binding affinity of pasireotide vs octreotide, it was hypothesized that the sstr5 vs sstr2 receptor activation ratio is the main driver of hyperglycemia after pasireotide. The results also suggest that stronger activation of sstr2 may counteract the hyperglycemic effect. Indeed, co-administration of octreotide, which has a high affinity for sstr2, with a hyperglycemic dose of pasireotide did not cause significant changes in plasma glucose levels. In conclusion, although pasireotide and octreotide inhibited insulin to a similar degree, only pasireotide administration was associated with hyperglycemia. The strong glucagon inhibitory effect exhibited by octreotide but not pasireotide may explain this observation. The lack of hyperglycemia during co-administration of pasireotide and octreotide may be explained by the greater activation of sstr2 compared with pasireotide alone, causing the insulin–glucagon balance to shift within the normoglycemic range. Extrapolation of these data to humans must account for species differences in islet cell sstr expression. Journal of Endocrinology (2012) 212, 49–60

Introduction

Somatostatin, an inhibitory hormone widely distributed throughout the central nervous system and peripheral tissues, plays an important regulatory role in neurotransmission and secretion, preventing the release of GH, TSH, gastrointestinal hormones, pancreatic enzymes and hormones, and neuroptides (Van Op den Bosch et al. 2009). After the discovery of somatostatin in 1973 (Brazeau et al. 1973), a new approach to the treatment of conditions associated with endocrine hypersecretion, such as acromegaly, Cushing’s disease, and symptoms of metastatic neuroendocrine tumors (NET), was initiated (Weckbecker et al. 2002).

Octreotide, a synthetic analog of somatostatin with more prolonged pharmacological actions than the endogenous hormone, has been in clinical use for the treatment of acromegaly and NET for more than 20 years. Octreotide primarily activates sstr2 and to a lesser extent sstr5 (Table 1). In the classical octreotide indications of acromegaly and NET, expression of somatostatin receptor subtypes sstr3 and sstr5 predominates, although the expression of multiple somatostatin receptor subtypes is common in most tissues expressing somatostatin receptors (Schmid 2008, 2010).

In other diseases associated with somatostatin receptors, the expression of the five receptor subtypes varies between tumor types. Indeed, ACTH-secreting pituitary adenomas predominantly express sstr5, followed by sstr2 and sstr1 (Hofland & Lamberts 2003). A somatostatin analog like pasireotide that can activate other somatostatin receptor subtypes besides sstr2 has the potential to be effective not only in patients with acromegaly or NET who respond to octreotide but also in patients unresponsive or refractory to octreotide, as well as in other diseases associated with somatostatin receptor expression besides sstr2, such as Cushing’s disease.

Pasireotide (SOM230) is a multireceptor-targeted somatostatin analog with high binding affinity for sstr1,2,3 and sstr5, including a 39- and 30-fold higher binding affinity for sstr5 and sstr1, respectively, than octreotide (Schmid & Schoeffter 2004; Table 1). Pasireotide showed a stronger inhibitory effect than octreotide on the secretion of GH, insulin-like growth factor 1 (IGF1), ACTH, and corticosterone in animal models (Bruns et al. 2002, Weckbecker et al. 2002, Schmid & Silva 2005, Silva et al. 2005), suggesting that pasireotide has the potential to be an effective therapy in patients with active acromegaly and Cushing’s disease who are not responsive to octreotide. Based on its high affinity to sstr5 and the strong
expression of sstr5 in corticotroph tumors, pasireotide might become the first medical treatment option for patients with Cushing’s disease. Indeed, pasireotide has demonstrated efficacy in a large randomized phase III trial in patients with Cushing’s disease (Colao et al. 2011) and therefore has the potential to be a novel pituitary-targeted treatment for patients with Cushing’s disease. In a phase II trial of 60 patients with acromegaly in which pasireotide was administered for 3 months, 27% of patients achieved biochemical control (GH ≤ 2.5 μg/l and IGF1 normalization) and almost 40% achieved significant tumor volume reduction (Peterson et al. 2010). In the 30 patients who continued receiving pasireotide as part of the extension phase, prolonged pasireotide treatment maintained biochemical control and maintained or increased tumor volume reduction (Farrall et al. 2011). Encouraging results have also been obtained in a phase II trial of 45 patients with functioning NET refractory to octreotide long-acting release (LAR) in which pasireotide provided improvement in the symptoms of carcinoid syndrome and was associated with stabilization of tumor growth (Kvols et al. 2010).

Somatostatin analogs have also been found to have an effect on glucose homeostasis in clinical trials, as expected based on the physiological effect of natural somatostatin. Glucose homeostasis is a complex process regulated by the interactions of various hormones, including insulin, glucagon, and somatostatin (among others), plus additional variables such as food intake, sleep, and physical exertion (Strowski et al. 2003, Drucker 2007). Somatostatin, an inhibitor of both insulin and glucagon secretion (Hauge–Evans et al. 2009), binds with high affinity to the sst1 receptor subtypes (Table 1), of which sstr2 and sstr5 are the predominantly expressed subtypes in human pancreatic islet cells. The inhibition of insulin is mediated mainly by sstr2 and sstr5 in humans and sstr4 in mice (Fagan et al. 1998, Zambre et al. 1999, Strowski et al. 2003), whereas the inhibition of glucagon is mediated almost entirely by sstr2 (Singh et al. 2007a,b) in both species.

To evaluate the effect of pasireotide on glucose homeostasis and possible mechanisms of action that might underlie acute hyperglycemia, a number of studies were undertaken in rats. Monkeys were not a suitable model as pasireotide does not increase plasma glucose at high effective doses in cynomolgus monkeys (Weckbecker et al. 2002), thus disqualifying this species (Macaca fascicularis) as being predictive of effects observed in humans. Results from studies evaluating glucose homeostasis in rats are reported here. These studies evaluated the effect of single and chronic s.c. injection of pasireotide and octreotide on glucose, insulin, glucagon, and/or IGF1 levels. Pasireotide was also administered as a continuous s.c. infusion and in a LAR formulation to evaluate the duration and persistence of the hyperglycemic effect. Studies in which octreotide and pasireotide were co-administered aimed to evaluate whether injection of octreotide would have an inhibitory effect on hyperglycemia induced by pasireotide administration.

The rationale for the co-application studies was based on the hypothesis that additional activation of sstr2 (mainly located on glucagon-producing α cells) would compensate for the stronger binding affinity of pasireotide for sstr5 (which are mainly located on insulin-producing β cells). This hypothesis is in line with the observation that octreotide has no hyperglycemic effect in rats, even at very high doses, despite the fact that octreotide has some affinity for sstr5 (Table 1). The fact that high doses of octreotide do not induce hyperglycemia indicates that increase in glucose levels is not caused by sstr5 activation alone. Furthermore, in co-application studies, due to the availability of higher concentrations of the agonists, the activation of sstr5 and sstr2, and the resulting inhibition of insulin, should be theoretically stronger, although the ratio of the relative activation of both receptor subtypes will be different from that seen in single-agent studies. As such, adding octreotide to a hyperglycemic dose of pasireotide should result in a more pronounced activation of sstr2 relative to sstr5, resulting in different ratios of insulin and glucagon release inhibition, due to the known receptor distribution on pancreatic islet cells.

### Materials and Methods

The experiments described in this paper were performed according to the national animal welfare requirements (Eidgenössische Tierversuchsbewilligung, Switzerland).

### Compounds and formulations

For all short-term experiments and for experiments in which pasireotide was applied s.c. by osmotic mini-pumps, pasireotide acetate was used. Pasireotide was dosed in 0.9% sterile saline and stocks were stored frozen in 1 mg/ml concentrations. On the day of experiment, the stock solutions were diluted with 0.9% sterile saline to the final concentration (pH 5–6). Octreotide acetate was prepared and stored (−20 °C) in the same way as pasireotide. For the long-term application of pasireotide and the long-term combination experiments with pasireotide and octreotide, the LAR formulations of this compound was used. Octreotide LAR...
saline and injected s.c. into the back of the rats. Each group consisted of six rats. For blood sampling, the animals were briefly anesthetized by inhalation of isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane) (5%, in air). Blood samples (~400 µl) were taken by sublingual bleeding prior to injection (t=0; i.e. 0700 h) and 0.25, 0.5, 1, 3, 6, and 8 h post-dose. Dosing started at 90 min after the end of the dark phase and was completed within 30 min for the entire cohort of each experiment. Plasma concentrations of glucose, pasireotide, and octreotide were determined from the same blood samples drawn at different time points for the PK/PD analysis (Fig. 1).

Blood samples from three individual short-term experiments were taken sublingually 1 h after the s.c. injection of saline, octreotide (10 µg/kg), or pasireotide (10 µg/kg; Fig. 2) and were analyzed for glucose, insulin, and glucagon (n=18 per group) to allow meaningful comparisons of these parameters at a time when the compounds were both present and showed their strongest effect on plasma glucose. Blood samples for the determination of glucose, insulin, and glucagon were collected in EDTA-coated tubes (TOM-14, Milian, Geneva, Switzerland) containing a general protease inhibitor cocktail (Sigma, P2714; 30 µl of 1:10 diluted stock solution/1 ml blood), which inhibits serine, cysteine, and metalloproteases with broad specificity.

**Single high-dose experiment** Adult male non-fasted Sprague–Dawley rats (OFA SD, Ifa Credo, Lyon, France) with a bodyweight of 300–340 g were used. Two groups of rats (n=4 each) received pasireotide or octreotide 500 µg/kg by s.c. injection in a volume of 1 ml/kg. Blood samples (~400 µl) were taken by sublingual bleeding 0.5, 1, 3, 6, and 24 h after dosing. Blood was collected in EDTA-coated tubes, kept at 4 °C, and centrifuged for 3 min at 19 300 g.

**Repeated dose experiment** Adult male Lewis rats (Wiga, Charles River, Sulzfeld, Germany), with a bodyweight of 300–330 g, were housed in type IV cages under controlled light (12 h light:12 h darkness; light from 0600 to 1800 h) at a temperature of 22 °C and were fed with water and a standard chow made available ad libitum. Two groups of rats (n=6 per group) were injected with a single s.c. dose of octreotide or pasireotide 10 µg/kg, or vehicle, at 0730 h. Measurements of glucose, insulin, and glucagon were performed from the same plasma samples obtained by sublingual bleeding of the animals 1 h after the injection. **P<0.01; ***P<0.001.

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**Figure 1** Free-feeding adult male Lewis rats (n=6 per group) were injected with a single s.c. dose of octreotide or pasireotide 1, 10, or 30 µg/kg; a) glucose levels and b) pasireotide and octreotide plasma concentration. P<0.0001 for the AUC of pasireotide 10 and 30 µg/kg vs vehicle using one-way ANOVA; Dunnett.

**Figure 2** Free-feeding adult male Lewis rats (n=6 per group) were injected with a single s.c. dose of octreotide or pasireotide 10 µg/kg, or vehicle, at 0730 h. Measurements of glucose, insulin, and glucagon were performed from the same plasma samples obtained by sublingual bleeding of the animals 1 h after the injection. **P<0.01; ***P<0.001.

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**Animals and treatments**

**Single low-dose experiment** Adult male non-fasted Lewis rats (Lew/Han/Hsd, Harlan, The Netherlands), with a bodyweight of 300–340 g, were housed in Makrolon type IV cages at 22 ± 2 °C and 40–70% humidity under a normal light–darkness cycle (light off 1800–0600 h) for at least 7 days. They were fed a standard chow (Provini 3890, from Kliba, Kaiseraugst, Switzerland) and had access to water made available ad libitum unless otherwise indicated. All animals were weighed on the day of dosing. Pasireotide acetate 1, 10, and 30 µg/kg; octreotide acetate 1, 10, and 30 µg/kg; and vehicle (saline only) were dissolved in sterile physiological saline and injected s.c. into the back of the rats. Each group consisted of six rats. For blood sampling, the animals were briefly anesthetized by inhalation of isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane) (5%, in air). Blood samples (~400 µl) were taken by sublingual bleeding prior to injection (t=0; i.e. 0700 h) and 0.25, 0.5, 1, 3, 6, and 8 h post-dose. Dosing started at 90 min after the end of the dark phase and was completed within 30 min for the entire cohort of each experiment. Plasma concentrations of glucose, pasireotide, and octreotide were determined from the same blood samples drawn at different time points for the PK/PD analysis (Fig. 1).

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but no blood samples were taken for glucose determination. Only on days 4–9, the study compound was injected, daily before and 1 h after drug administration. The light–darkness cycle in the animal room was from 1800 to 0600 h. *P<0.05.

each) received twice-daily s.c. injections of pasireotide or octreotide 10 µg/kg or vehicle for 12 days (Fig. 3). The two daily injections were administered 6 h apart. The first injection was at 0700 h, i.e. 1 h after lights on each day. Blood samples (~100 µl) were taken by sublingual bleeding immediately before and 1 h after each dose on days 1, 2, 3, 10, 11, and 12. For blood sampling, the animals were briefly anesthetized by inhalation of isoflurane (5%, in air). Blood was collected in EDTA-coated tubes, kept at 4 ºC, and centrifuged for 3 min at 19 300 g. Plasma samples were divided and immediately frozen at −25 ºC until analysis of glucose. Only on days 4–9, the study compound was injected, but no blood samples were taken for glucose determination.

Continuous s.c. infusion experiment Five groups (n = 6 each) of male Lewis rats (Lew/Han/Hsd), 300–350 g, were treated with sterile saline, octreotide 3 and 10 µg/kg per h, or pasireotide 3 and 10 µg/kg per h using osmotic mini-pumps (Alzet, Model 2ML2), which continuously released the compounds s.c. at a rate of 0.5 µl/h for >14 days (Table 2 and Fig. 4). Mini-pumps were filled and inserted according to the manual of the provider. Rats were briefly anesthetized with isoflurane (3%, in air) and mini-pumps implanted s.c. on their backs. All rats received 0.03 mg/kg buprenorphine (Temgesic, Basel, Switzerland) as analgesic immediately before surgery. Pasireotide and octreotide were dissolved in distilled water and applied in a dose of 10 µg/kg per h. Blood samples (~400 µl) were taken by sublingual bleeding before surgery and on days 1, 7, and 14 after mini-pump implantation. Blood was collected between 0900 and 1100 h on each sampling day and treated as described above.

LAR formulation experiments Both pasireotide LAR and octreotide LAR (4 and 8 mg/kg active compound) were injected s.c. in male Lewis rats (Lew/Han/Hsd) weighing 270–290 g (n = 6 per group). Blood was taken sublingually between 0800 and 1000 h on days −1, 1, 4, 10, 15, 20, 25, 30, and 35, as described above (Figs 5 and 6). Glucose, insulin, glucagon, and IGF1 were determined from blood samples collected in EDTA-coated tubes containing protease inhibitor cocktail (Sigma, P-2714) and kept on ice until preparation of plasma by centrifuging at 15 000 g for 10 min at 4 ºC. Plasma samples were stored at −20 ºC until analysis.

Pasireotide and octreotide combination experiments For short-term combination experiments, 10 µg/kg pasireotide acetate and octreotide acetate were injected s.c. in adult male non-fasted Lewis rats (290–320 g, n = 6 per group) between 0800 and 1000 h on the experimental day, and blood samples were collected 15, 30, and 60 min after the injection (Fig. 7). Glucose, insulin, and glucagon were determined from sublingually collected blood samples as described below.

For long-term combination experiments, pasireotide LAR and octreotide LAR (8 mg/kg active substance) were injected once s.c. in adult male Lewis rats (270–300 g, n = 6 per group) between 0900 and 1000 h on the experimental day, and blood samples were collected on days −1, 2, 5, 10, 15, 20, 25, 29 (or 30), and 35 after the injection for the determination of changes in glucose. Insulin, glucagon, and IGF1 levels were determined from sublingually collected blood samples drawn on days −1, 1, 5, 15, 20, 25, and 35. In contrast to the single-compound experiments, which continued up to 35 days after only one injection, the application of the first injection of pasireotide LAR was followed by a second injection of octreotide LAR on day 16. Conversely, those rats that received octreotide LAR first were treated with pasireotide LAR on day 16. Thus, it was possible to obtain baseline values for the first 15 days and subsequently the effect of the combination treatment from the same animals. Control animals received an injection of vehicle on days −1 and 16.

Table 2 Plasma concentrations of pasireotide and octreotide in male Lewis rats during 14 days’ continuous s.c. infusion by osmotic mini-pump

<table>
<thead>
<tr>
<th>Day</th>
<th>Pasireotide 3 µg/kg per h n = 6</th>
<th>Pasireotide 10 µg/kg per h n = 6</th>
<th>Octreotide 3 µg/kg per h n = 6</th>
<th>Octreotide 10 µg/kg per h n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.02 ± 1.53</td>
<td>12.98 ± 4.73</td>
<td>2.17 ± 0.38</td>
<td>5.780 ± 1.14</td>
</tr>
<tr>
<td>7</td>
<td>7.07 ± 1.11</td>
<td>8.88 ± 2.34</td>
<td>1.53 ± 0.78</td>
<td>4.554 ± 0.94</td>
</tr>
<tr>
<td>14</td>
<td>8.63 ± 1.04</td>
<td>17.33 ± 10.55</td>
<td>2.07 ± 0.63</td>
<td>7.530 ± 0.66</td>
</tr>
</tbody>
</table>
Analytical methodology

Blood glucose concentration from the acute short-term and long-term studies were determined from full blood using the digital Accu-Chek compact (Roche Diagnostics) with integrated test strips. The instrument needs only 1.5 µl blood and has a measuring range of 0.6–33.3 mmol/l (10–600 mg/dl) of glucose. The instrument was calibrated before every experiment. Glucose measurements from the 12-day repeated-dose study were performed in plasma collected from non-fasted rats on a COBAS MIRA S chemistry analyzer (Roche Diagnostics) using the enzymatic quantitative Glucose Hexokinase Test (cat. no. 1447513, lot 641 360-01) from Roche/Hitachi. The measuring range of the test for serum/plasma is 0.11–41.6 mmol/l.

IGF1 levels were determined, as described previously (Schmid & Silva 2005), from 25 µl plasma using a commercial ELISA (Immunodiagnostics Systems Ltd; IDS, Bolden, UK, cat. no. AC-18F1) according to the instructions of the supplier.

Insulin and glucagon were measured simultaneously in the same blood sample (25 µl) using multiplex analytics of bead-based assays (Lincoplex, Millipore, Zug, Switzerland, Kit RENDO–85K–02). The assay kit was applied according to the instructions of the supplier and had a detection limit of 0.022 ng/ml for glucagon and 0.036 ng/ml for insulin.

Pasireotide plasma concentrations were determined by HPLC/tandem mass spectrometry (MS–MS). Briefly, following chromatographic separation, the column eluent was directly introduced into the ion source of the ion-trap mass spectrometer XCT Agilent (Agilent, Basel, Switzerland), controlled by Agilent ChemStation (LC 3D system) software. Electrospray-positive ionization (ESI+) single-ion recording was used for the MS–MS detection of the analyte. The fragmented quasimolecular ions [MHH]2+ at m/z 524.40 for pasireotide and m/z 539.40 for the internal standard were used. The limit of quantification (LOQ) was set to 0.4 ng/ml. Regression analysis was performed using ChemStation (Agilent) and Excel 2002 (Microsoft) software. Concentrations of unknown samples were back-calculated based on the peak area ratios of analyte/IS from the calibration curve constructed using calibration samples spiked in blank plasma obtained from untreated (not dosed) animals. Assay linearity was indicated by an overall regression coefficient of 0.9959. For the quantification of octreotide, the MS assay was modified accordingly, using the same internal standard.

Areas under the plasma concentration vs time curves (AUC) were calculated from the mean values using the linear trapezoidal rule, applying a non-compartmental model for bolus i.v. or oral dosing respectively (WinNonlin Version 4.0, Pharsight). The terminal elimination half-life (t1/2) was calculated using the same model.

Statistical analysis

Results are expressed as mean ± S.E.M. Data obtained at each time point were compared between groups via ANOVA followed by a Tukey paired t-test using the program GraphPad Prizm 3.03, San Diego, CA, USA.

Figure 4 Effect of 14 days’ continuous s.c. infusion by osmotic mini-pump of pasireotide and octreotide 3 and 10 µg/kg per h on plasma a) glucose, b) insulin, c) glucagon, and d) IGF1 levels in male Lewis rats (n=6 per group). MP, mini-pump. *P<0.05 vs vehicle control.
Results

Acute effects of low-dose pasireotide on plasma glucose

In the single low-dose experiment, pasireotide 10 and 30 μg/kg, but not pasireotide 1 μg/kg, caused a rapid and transient increase in plasma glucose levels in free-feeding rats when compared with vehicle (Fig. 1a). By contrast, octreotide 1, 10 and 30 μg/kg had no effect on glucose (Fig. 1a). In rats treated with pasireotide 10 and 30 μg/kg, mean plasma glucose levels increased from a baseline of 7.9 and 7.8 mmol/l to a peak of 13.3 and 13.2 mmol/l, respectively, at 3 h (P<0.001 vs baseline and vehicle). By 8 h post-dose, glucose levels in rats administered pasireotide 10 and 30 μg/kg had decreased to near baseline levels of 8.2±0.5 and 9.0±0.3 mmol/l respectively.

After a single injection of pasireotide 10 or 30 μg/kg, peak plasma concentrations (Cmax) of pasireotide (7.6±0.4 and 18.2±1.9 ng/ml respectively) were reached within 1 h (Fig. 1b). Comparing the PK with the PD profile on glucose revealed that the plasma glucose level decayed more slowly than the plasma concentration of pasireotide. For example, 6 h after the application of the pasireotide 30 μg/kg dose, the plasma concentration of pasireotide was already reduced to ~66% of its peak levels at 1 h; however, the plasma glucose concentration was only slightly lower at 6 h compared with its peak value at 3 h. Subcutaneous octreotide and pasireotide 1 μg/kg did not result in measurable plasma concentrations at any time point. Interestingly, 15 min after the injection of octreotide and pasireotide 10 and 30 μg/kg, both compounds were present in the plasma at similar concentrations. Thereafter, the plasma concentration of octreotide declined more rapidly than pasireotide and was below LOQ between 1 and 3 h, whereas pasireotide could still be determined 8 h post-dose. The t1/2 of pasireotide and octreotide was 3.6 h and 20 min, respectively, after a 30 μg/kg dose and, thus, 11-fold longer for pasireotide than for octreotide. Similarly, an eightfold longer plasma half-life was obtained for pasireotide when comparing the respective t1/2 of pasireotide and octreotide after injection of lower doses (10 μg/kg).

Injection of the vehicle saline or octreotide in any dose (1, 10, 30 μg/kg s.c.) resulted in a similar small and transient drop in plasma glucose, which lasted 1–3 h. By contrast, the injection of two higher doses of pasireotide (10 and 30 μg/kg s.c.) resulted in a rapid increase in plasma glucose, which peaked at 1–3 h and lasted up to 6 h. The increase in plasma glucose was very rapid and reached statistical significance 15 min post-dose. Although the lowest dose of pasireotide did not result in measurable plasma concentrations at any time point, the elevation in plasma glucose was statistically significant 6 h post-dose and, thus, 11-fold longer for pasireotide than for octreotide when comparing the respective t1/2 of pasireotide and octreotide at any time point. Interestingly, 15 min after the injection of octreotide and pasireotide 10 and 30 μg/kg, both compounds were present in the plasma at similar concentrations. Thereafter, the plasma concentration of octreotide declined more rapidly than pasireotide and was below LOQ between 1 and 3 h, whereas pasireotide could still be determined 8 h post-dose. The t1/2 of pasireotide and octreotide was 3.6 h and 20 min, respectively, after a 30 μg/kg dose and, thus, 11-fold longer for pasireotide than for octreotide. Similarly, an eightfold longer plasma half-life was obtained for pasireotide when comparing the respective t1/2 of pasireotide and octreotide after injection of lower doses (10 μg/kg).

Additional observations obtained in this study included a slight increase in plasma IGF1 levels after treatment with the vehicle saline or octreotide in any dose (1, 10, 30 μg/kg s.c.) and a similar small and transient increase after injection of pasireotide 10 and 30 μg/kg s.c. (Fig. 2).}

Figure 5 Effects of pasireotide LAR and octreotide LAR 4 and 8 mg/kg on plasma a) glucose, b) insulin, and c) glucagon levels in male Lewis rats (n=6 per group) during 35 days of treatment. For clarity, the effects of both compounds in this study are displayed separately with the same vehicle control. *P<0.05 vs vehicle control using one-way ANOVA Dunnett.

Figure 6 Effects of pasireotide LAR and octreotide LAR 4 and 8 mg/kg on plasma IGF1 levels in male Lewis rats (n=6 per group) during 35 days of treatment. The IGF1 values were determined from the experiment used to obtain the data displayed in Fig. 5. *P<0.05 vs vehicle control using one-way ANOVA Dunnett.
not result in measurable plasma levels at any time, the glucose concentration 10–60 min after dosing was slightly, but significantly, elevated compared with the vehicle control at the same time points. This indicates a higher sensitivity of the glucose-elevating effect of pasireotide (pharmacodynamic readout) compared with the compound detection assay (pharmacokinetic measurement).

Blood levels of glucose, insulin, and glucagon were determined in rats 1 h after administration of pasireotide and octreotide 10 μg/kg, i.e. at a time when both compounds were present at concentrations of 2–18 ng/ml in the blood. At this dose, pasireotide caused a highly significant \( (P<0.001) \) increase in glucose from 7.1 ± 0.2 to 11.4 ± 0.4 mmol/l, whereas octreotide caused no hyperglycemic effect (6.6 ± 0.2 mmol/l; Fig. 2). Plasma insulin levels were not significantly different in control vs octreotide- or pasireotide-treated rats. By contrast, glucagon levels were strongly and significantly reduced by octreotide (0.03 ± 0.01 ng/ml) and, to a lesser extent, pasireotide (0.11 ± 0.02 vs 0.21 ± 0.03 ng/ml for vehicle-treated controls). In this study, plasma levels of glucose and insulin 1 h after saline injection were lower than in subsequent studies (Figs 4 and 5), where pasireotide caused statistically significant reductions in plasma insulin levels.

**Acute effects of high-dose pasireotide on plasma glucose**

Qualitatively similar results were seen after a single high dose of pasireotide or octreotide 500 μg/kg s.c. in free-feeding male Sprague-Dawley rats. Plasma glucose increased from baseline values of 11.1 mmol/l to a peak of 21.2 mmol/l 3 h after administration of a high dose of pasireotide. Plasma glucose levels then decreased to the baseline value by 24 h. Octreotide had a negligible effect on plasma glucose following a single high dose. The \( C_{max} \) of pasireotide (461 ± 1 ± 65.49 ng/ml) was seen at 1 h, whereas the \( C_{max} \) of octreotide (152 ± 37.5 mmol/l) was observed at 0.5 h.

The calculated elimination \( t_\text{e} \) was 8.5 ± 1.3 h for pasireotide and 0.7 ± 0.1 h for octreotide. The \( t_{max} \) for both compounds was 1 h, and \( C_{max} \) was significantly higher for pasireotide (461 ± 66 ng/ml) than for octreotide (173 ± 21 ng/ml). The AUC was ninefold higher with pasireotide (2326 ± 323 ng × h/ml) than with octreotide (273 ± 13 ng × h/ml), and the calculated clearance of pasireotide was 226 ± 27 ml/h per kg, compared with 1843 ± 84 ml/h per kg for octreotide. These data confirm the superior bioavailability of pasireotide vs octreotide in rats, as previously demonstrated in patients with acromegaly (Ma *et al.* 2005).

**Effects of repeated s.c. application on plasma glucose**

During 12 days of twice-daily administration, the vehicle-treated control group showed only minimal fluctuations in plasma glucose concentrations, as demonstrated by the detailed graphical representation shown in Fig. 3. Statistically significant and biologically meaningful increases in plasma glucose vs the respective control values were observed for pasireotide 10 μg/kg on only days 1 and 2. During the last 3 days of treatment, although plasma glucose levels were statistically different from vehicle, these differences were not considered to be physiologically relevant because the overall changes were small and were predominantly caused by injection of the vehicle rather than resulting from an effect of pasireotide. Compared with the first 3 days of treatment, the smaller reduction of plasma glucose observed with octreotide 10 μg/kg on the last 3 days of treatment was equally observed in the vehicle-treated group. The second daily application of pasireotide, 6 h after the first, consistently resulted in a smaller increase in glucose than the first application. By contrast, octreotide injections caused small and transient reductions in plasma glucose levels (Fig. 3).

**Effects of continuous s.c. infusion on plasma glucose, insulin, glucagon, and IGF1**

Continuous s.c. infusion of pasireotide caused elevated plasma glucose levels compared with vehicle-treated controls, which reached statistical significance only on days 1 and 7 in the pasireotide groups 3 and 10 μg/kg per h respectively (Fig. 4a). After 14 days of treatment, no statistically significant difference in plasma glucose levels was observed between rats treated with pasireotide or vehicle. Glucose levels in octreotide-treated rats were lower at all time points compared with vehicle-treated controls and reached statistical significance on days 1 and 14. Only rats treated with octreotide 10 μg/kg per h showed a statistically significant reduction on days 1 and 7 (Fig. 4b). However, octreotide strongly inhibited glucagon release, whereas the inhibitory effect of pasireotide on glucagon secretion was negligible (Fig. 4c). In the same experiment, plasma IGF1 was similarly inhibited continuously for 14 days by both doses of pasireotide to 55 and 60% of control values, which were at 1123 ± 48 ng/ml in vehicle-treated controls (Fig. 4d). By contrast, octreotide had only
a transient inhibitory effect on IGF1, which did not reach statistical significance despite its strong and long-lasting inhibitory effect on glucagon. In line with the stronger inhibition of IGF1 was the stronger effect of pasireotide on bodyweight. Bodyweight of vehicle-treated control rats increased 20% from 262±5 to 314±5 g during the 14-day treatment period. By contrast, rats treated with octreotide 3 and 10 μg/kg per h gained only 10 (NS) and 8% (P<0.001), respectively, during this time. Pasireotide prevented bodyweight gain and caused a slight reduction in bodyweight of 3% (P<0.001) by day 14 with both doses. Plasma levels of both compounds infused by osmotic mini-pumps remained stable throughout the 14-day treatment period, and relatively similar values were obtained for pasireotide 3 μg/kg per h and octreotide 10 μg/kg per h (Table 2).

Effects of a single LAR dose on plasma glucose, insulin, glucagon, and IGF1

Qualitatively similar results were observed following a single dose of pasireotide LAR 4 or 8 mg/kg and octreotide LAR 4 or 8 mg/kg. Despite a transient elevation of glucose after pasireotide on day 1, neither drug at either dose had a significant effect on plasma glucose in rats after day 5 (Fig. 5a). The increased glucose level observed in the vehicle group on day 5 may be due to increased food intake and a subsequent rise in insulin levels after the animals woke up. The inhibitory effect of pasireotide on insulin secretion may explain the absence of this peak in glucose levels in the pasireotide–treated groups. Plasma insulin was inhibited similarly by both compounds (Fig. 5b). Glucagon levels were strongly and persistently inhibited by a single injection of octreotide LAR for more than 35 days, whereas pasireotide LAR caused only a moderate inhibitory effect on glucagon on day 1 (Fig. 5c).

The inhibitory effect of pasireotide LAR on IGF1 was markedly stronger than that of octreotide LAR (Fig. 6). Pasireotide LAR 4 and 8 mg/kg caused significant and long-lasting reductions in plasma IGF1 ≥35 days, whereas octreotide LAR 4 and 8 mg/kg significantly inhibited IGF1 secretion on day 1, and octreotide LAR 4 mg/kg on day 35. Bodyweight during treatment with pasireotide LAR (4 and 8 mg/kg) remained stable over 35 days, whereas rats treated with vehicle and octreotide LAR (8 mg/kg) gained 35 and 26% in bodyweight respectively.

Effects of co-application of pasireotide and octreotide on plasma glucose, insulin, and glucagon

In contrast to the single-agent experiments, which continued up to 35 days after only one injection, the application of the first injection of pasireotide LAR was followed by a second injection of octreotide LAR on day 16. Conversely, those rats that received octreotide LAR first were treated with pasireotide LAR on day 16. Thus, both compounds were present in both treatment groups from days 16–35.
After administration of pasireotide LAR on day 1, glucose levels showed a rapid initial rise, followed by a gradual decline to day 10, but remained above the respective control levels as long as pasireotide was the only compound present on day 15 (Fig. 9). After additional administration of octreotide LAR on day 16 in the same animals, glucose levels decreased significantly from 8.3 mmol/l on day 15 to 6.6 mmol/l on day 20 ($P<0.001$), and the values remained below the respective control values for the remaining time period until day 35. In rats that received octreotide LAR followed by pasireotide LAR, glucose levels remained below those of control animals on six out of eight experimental days and stayed relatively constant throughout the 35-day treatment period (Fig. 9).

Plasma insulin levels were inhibited to a similar extent during the first 15 days by pasireotide LAR and octreotide LAR (Fig. 10), whereas glucagon levels were inhibited to a greater extent with octreotide LAR. After co-application with either octreotide LAR or pasireotide LAR on day 16, both plasma insulin and glucagon levels were inhibited to a similar extent in both treatment groups (Fig. 10).

Discussion

A single s.c. injection of pasireotide elevated plasma glucose rapidly and dose dependently in rats, whereas octreotide had no such hyperglycemic effect. The observed elevation of plasma glucose levels seen with pasireotide was transient and showed rapid tachyphylaxis after repeated s.c. injections and even more so during constant infusion of the compound using osmotic mini-pumps or the administration of the long-acting LAR formulation. Pasireotide and octreotide showed similar inhibitory effects on insulin, whereas the inhibitory effect of octreotide on glucagon was stronger than that of pasireotide. Co-administration of octreotide and pasireotide completely inhibited the pasireotide-induced increase in glucose.

Somatostatin and its analogs have general inhibitory effects on hormone secretion (Norman et al. 2002). Previous studies on cultured murine and human islet cells showed that the inhibitory effects of somatostatin on insulin secretion are mediated predominantly by sstr5 (Zambre et al. 1999, Strowski et al. 2003, Tirone et al. 2003). However, involvement of sstr2 in insulin inhibition is also likely, given the expression of both sstr2 and sstr5 on islet $\beta$ cells. Furthermore, glucagon inhibition is mediated primarily by sstr2 (Singh et al. 2007a,b). Therefore, the different effects of pasireotide and octreotide on insulin secretion and glucose homeostasis seen in the current studies may be explained by the different somatostatin receptor subtype affinities and functional activities of the two somatostatin analogs and by the difference in somatostatin receptor subtype distribution on the rodent $\alpha$ and $\beta$ cells. In rats, the expression of sstr2 seems to be exclusive for $\alpha$ cells and expression of sstr5 for $\beta$ cells (Mitra et al. 1999, Zambre et al. 1999, Strowski et al. 2003). In human pancreatic islets, receptor subtype expression seems to be more varied. Using double-label immunohistochemistry, sstr1, sstr2, and sstr5 were expressed on 100, 44, and 87% of $\beta$ cells, respectively, and on 26, 89, and 35% of $\alpha$ cells respectively. Expression of sstr3 was only occasionally observed and sstr4 was absent in human islet cells (Kumar et al. 1999). Thus, despite the differences in receptor subtype expression in rat and human islet cells, the predominant expression of sstr5 on $\beta$ cells and sstr2 on $\alpha$ cells was found in both species. Furthermore, a functional study on isolated human islet cells confirmed the importance of the sstr3 receptor for insulin secretion (Zambre et al. 1999). In studies on healthy human subjects, infusion of natural somatostatin results in a transient increase in glucose for about 6 h, despite a sustained suppression of insulin and glucagon (Rizza et al. 1979).

Pasireotide has a 39-fold higher binding affinity and a 158-fold higher functional activity at sstr3 than octreotide and a twofold lower affinity and a sevenfold lower functional activity at sstr2 (Bruns et al. 2002, Schmid & Schoeffter 2004). The transient hyperglycemia after acute s.c. administration of pasireotide in rats is in line with its stronger inhibition of insulin (via sstr5) relative to glucagon (via sstr2). Similarly, the small hypoglycemic effect observed after acute and long-term administration of octreotide in rats is in line with the observed stronger inhibition of glucagon vs insulin after administration of octreotide. In long-term studies, the hyperglycemic effect of pasireotide was small due to rapid tachyphylaxis, as can be concluded from the fact that the same plasma concentration of pasireotide (12–18 ng/ml), which resulted in maximal hyperglycemia in acute studies, had no effect on plasma glucose in long-term studies. The small residual hyperglycemia after prolonged pasireotide administration can be explained by the small-to-moderate inhibition of insulin and lack of inhibition of glucagon. Similar results were seen with pasireotide LAR, which releases pasireotide over a
period of at least 28 days. Pasireotide LAR had only minimal effects on plasma glucose in rats after day 5, despite its presence in plasma, which caused marked hyperglycemia after acute administration. Although sstr1 receptor subtypes are also expressed in rat and human pancreatic islets, their contribution must be regarded as negligible based on our data showing that octreotide could inhibit the pasireotide-induced hyperglycemia, although it has only a very low affinity for this receptor subtype.

The results obtained with pasireotide LAR provide further evidence that the elevation of plasma glucose seen with pasireotide is an acute effect and that longer term administration leads to an attenuation of the hyperglycemic effect. In the combination studies, by adding octreotide LAR after 15 days of pasireotide LAR, the pasireotide-induced increase in glucose was completely inhibited, implying that the insulin–glucagon balance was shifted to levels that are in line with slightly lower plasma glucose. Although other (possibly unknown) factors that might contribute to this shift cannot be excluded, the differences in insulin and glucagon balance caused by pasireotide and octreotide are in line with the currently available data. The inhibition of glucagon by octreotide, which has a higher binding affinity to sstr2 on pancreatic α cells, was slightly higher and counterbalanced the inhibition of insulin by pasireotide, which has a higher binding affinity to sstr3 on β cells; thus, an elevation in blood plasma glucose levels was not observed with combination therapy.

Previous studies on patients with acromegaly showed that octreotide and lanreotide reduce insulin resistance but simultaneously impair insulin secretion, with the overall balance being a slight deterioration in glucose homeostasis in non-diabetic patients with acromegaly (Baldelli et al. 2003, Steffin et al. 2006). In a phase II study of 15 days’ treatment with twice-daily s.c. administration of pasireotide 600 μg in patients with Cushing’s disease, hyperglycemia was reported in 14 of 39 patients (Boscaro et al. 2009). In addition to the general metabolic imbalance of these patients, individual differences in the somatostatin receptor expression profile on pancreatic α and β cells may underlie this response (Kumar et al. 1999). However, consistent with the results from the current animal studies, the increases in blood glucose in patients with Cushing’s disease occurred with initial exposure to pasireotide, generally improved to near baseline values 8 h after the s.c. injection, and were in general less pronounced after the first day of treatment, suggesting an attenuation of effect over time. Moreover, the observed increases in fasting blood glucose were more notable in patients with a history of impaired fasting blood glucose or type 2 diabetes mellitus prior to receiving pasireotide.

Currently available somatostatin analogs have a well-characterized IGF1 inhibiting action. Previous pre-clinical studies have found that pasireotide achieves greater and longer suppression of IGF1 levels than octreotide (Weckbecker et al. 2002, Schmid & Silva 2006), and our own findings support this result. Furthermore, in patients with acromegaly, pasireotide achieved significant reduction in IGF1 levels (Petersenn et al. 2010). The potential impact of plasma IGF1 levels on glucose homeostasis is complex. Targeted ablation of IGF1 receptors in pancreatic β cells in mice has been shown to impair glucose-induced insulin secretion (Xuan et al. 2002), suggesting that IGF1 may have a role in the stimulation of insulin secretion, with lower IGF1 levels potentially...
associated with lower insulin output. The potential impact of IGF1 reduction in models treated with somatostatin analogs is difficult to isolate, however, as these agents have a direct effect on sstr. Patients with acromegaly treated with pegvisomant, an agent that lowers IGF1 levels through an sstr-independent mechanism, may be a good model to explore this effect. In a study of patients with acromegaly switched from octreotide LAR to pegvisomant, glycemic control improved during pegvisomant therapy (Barkan et al. 2005). However, the fact that improvements in glucose metabolism during pegvisomant therapy were observed regardless of whether IGF1 levels were within the normal range during octreotide LAR therapy may indicate that IGF1 levels alone were not the main driver of this effect.

The mechanism behind hyperglycemia associated with pasireotide has been explored in two recent studies of healthy volunteers (Henry et al. 2011). First, the effect of pasireotide 600 or 900 μg s.c. bid on insulin secretion, hepatic sensitivity to insulin, and peripheral insulin sensitivity was assessed in a double-blind, randomized, single-center trial using the hyperglycemic clamp test to quantify insulin secretion and the hyperinsulinemic–euglycemic clamp test to evaluate insulin sensitivity or resistance. Results indicated that the main mechanism of pasireotide-induced hyperglycemia is secondary to a marked inhibition of insulin and incretin secretion (both GLP1 and GIP), with minimal inhibition of glucagon secretion and no impact on insulin sensitivity. In the second healthy volunteer trial, various antidiabetic agents were used in combination with pasireotide to assess the impact of co-application on glucose and insulin levels compared with those during pasireotide monotherapy. Results of this study suggested that incretin-based antihyperglycemic agents such as GLP-1 analogs (e.g. liraglutide) and DPP4 inhibitors (e.g. vildagliptin), followed by insulin secretagogues (e.g. nateglinide), may be able to ameliorate the effects of pasireotide on glucose homeostasis. The finding in our study that co-application of octreotide LAR eliminated pasireotide LAR-induced hyperglycemia is of potential therapeutic utility. Evaluation of this regimen in human subjects may be of potential interest as an alternative to co-application of pasireotide LAR with an antidiabetic medication. Theoretically, an octreotide LAR + pasireotide LAR combination therapy may offer the advantage of providing higher plasma values of both compounds, leading to greater therapeutic utility. In addition, this combination may allow for a favorable safety profile as octreotide LAR has shown an excellent tolerability even at high doses (Pleseriu 2011), and data available for pasireotide suggest a good tolerability profile once the hyperglycemia aspect has been corrected for (Colao et al. 2011).

In conclusion, pasireotide had a stronger inhibitory effect on insulin than on glucagon in rats, which may account for the transient hyperglycemia observed after acute s.c. injection of pasireotide. Conversely, the stronger inhibition of glucagon vs insulin seen with octreotide may explain the normoglycemia or the small hypoglycemic effect seen after acute administration of octreotide in rats. Glucose levels in rats following repeated administration of pasireotide normalized quickly, suggesting an attenuation of effect over time, and twice-daily administration of pasireotide did not result in an additive elevation of glucose. When octreotide LAR was administered to rats 16 days after administration of pasireotide LAR in the same animals, the pasireotide-induced increase in glucose was completely inhibited, suggesting that the insulin–glucagon balance was shifted to levels that are in line with slightly lower plasma glucose. Long-term phase III studies comparing pasireotide with octreotide in patients with acromegaly and metastatic NET are ongoing, as is a long-term phase III study on Cushing’s disease; these studies will help to further determine the effects of somatostatin analogs on glucose homeostasis in a clinical setting.

Declaration of interest

Both H S and J B are employees of Novartis Pharma AG, Basel, Switzerland.

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