EXPERIMENTAL STUDY

The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5

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Abstract

Objective: Currently, there is no effective medical treatment for patients with pituitary-dependent Cushing’s disease. A novel somatostatin (SS) analogue, named SOM230, with high binding affinity to SS receptor subtypes sst1, sst2, sst3 and sst5 was recently introduced. We compared the in vitro effects of the sst2-preferring SS analogue octreotide (OCT) and the multi-ligand SOM230 on ACTH release by human and mouse corticotroph tumour cells.

Methods: By quantitative RT-PCR the sst subtype expression level was determined in human corticotroph adenomas. In vitro, the inhibitory effect of OCT and SOM230 on ACTH release by dispersed human corticotroph adenoma cells and mouse AtT20 corticotroph adenoma cells was determined. In addition, the influence of dexamethasone on the responsiveness to OCT and SOM230 was studied.

Results: Corticotroph adenomas expressed predominantly sst5 mRNA (six out of six adenomas), whereas sst2 mRNA expression was detected at significantly lower levels. In a 72 h incubation with 10 nmol/l SOM230, ACTH release was inhibited in three out of five cultures (range 230 to 240%). Ten nmol/l OCT slightly inhibited ACTH release in only one of five cultures (228%). In AtT20 cells, expressing sst2, sst3 and sst5, SOM230 inhibited ACTH secretion with high potency (IC50 0.2 nmol/l). Dexamethasone (10 nmol/l) pre-treatment did not influence the sensitivity of the cells to the inhibitory effect of SOM230, suggesting that sst5 is relatively resistant to negative control by glucocorticoids.

Conclusions: The selective expression of sst5 receptors in corticotroph adenomas and the preferential inhibition of ACTH release by human corticotroph adenoma cells by SOM230 in vitro, suggest that SOM230 may have potential in the treatment of patients with pituitary-dependent Cushing’s disease.

European Journal of Endocrinology

Introduction

The first choice of treatment of patients with pituitary-dependent Cushing’s disease is surgery. If surgery fails, radiotherapy, alone or in combination with steroidogenic inhibitors, may be used (1, 2). These secondary options are primarily due to the absence of an effective medical treatment option (3, 4). Some preliminary data suggest a potential use of dopamine agonists, alone or in combination with ketoconazole, in selected cases of Cushing’s disease or Nelson’s syndrome (2). The expression of somatostatin (SS) receptors (sst) in adrenocorticotrophin (ACTH)-secreting pituitary adenomas has been studied in vivo and in vitro. In vivo, none of a series of eight corticotroph microadenomas showed an increased uptake of 111In-DTPA-labelled octreotide (OCT), whereas sst scintigraphy was positive in two invasive ACTH-secreting macroadenomas and two cases of Nelson’s tumours (5). The current generation of sst2-prefential SS analogues have no suppressive effect on ACTH level in patients with untreated Cushing’s disease, who have elevated cortisol levels (6). However, the SS analogue OCT suppressed pathological ACTH release in patients with Nelson’s syndrome and ACTH and cortisol secretion in patients with Cushing’s syndrome caused by ectopic ACTH secretion (7–9). Overall, these data suggest that in corticotroph adenomas of untreated patients with Cushing’s disease levels of sst2, one of the five known sst subtypes to which OCT binds preferentially, are low, and that this receptor...
subtype may be downregulated when circulating cortisol levels are high. The fact that ectopic ACTH-secreting tumours express sufficient sst numbers allowing their in vivo visualization by sst scintigraphy and responsiveness to glucocorticoids, compared with corticotroph adenomas (10). Additional in vitro evidence for a sst downregulation by hypercortisolaemia comes from studies using primary cultures of human corticotroph adenomas, in which it was shown that glucocorticoids downregulated the response of corticotrophin-releasing hormone (CRH)-induced ACTH secretion to OCT (11). Little is known yet about the quantitative expression levels of the five sst subtypes (sst1–5) in normal and tumoral corticotroph cells. By double-labelling in situ hybridization analysis for sst2 and sst5 mRNA, Day et al. (12) showed that normal rat corticotrophs expressed preferentially sst5 mRNA. In a few selected cases expression of sst subtype mRNA transcripts was studied in human corticotroph adenomas. In these studies it was found that the highest frequency of expression of sst mRNA transcripts was found for sst2 and sst5, and was found that the highest frequency of expression of human corticotroph adenomas. In these studies it was found that the highest frequency of expression of sst mRNA transcripts was found for sst2 and sst5, and was found that the highest frequency of expression of sst mRNA transcripts was found for sst2 and sst5, and was found that the highest frequency of expression of sst mRNA transcripts was found for sst2 and sst5. Little is known yet about the quantitative expression levels of the five sst subtypes (sst1–5) in normal and tumoral corticotroph cells. By double-labelling in situ hybridization analysis for sst2 and sst5 mRNA, Day et al. (12) showed that normal rat corticotrophs expressed preferentially sst5 mRNA. In a few selected cases expression of sst subtype mRNA transcripts was studied in human corticotroph adenomas. In these studies it was found that the highest frequency of expression of sst mRNA transcripts was found for sst2 and sst5, and to a lesser extent sst1 (13–16). Although sst2 mRNA can be found in corticotroph adenomas, their levels of expression are apparently low since OCT has no effect on ACTH secretion in patients with pituitary-dependent Cushing’s disease. However, at present the role of the other frequently expressed sst subtype, sst5, on ACTH secretion by corticotroph adenoma cells is unclear.

Recently, a novel multi-ligand SS analogue, SOM230, has been synthesized. Compared with OCT, SOM230 has a 30-, 5- and 40-times higher binding affinity for sst1, sst3 and sst2 receptors respectively, and 2.5-times lower affinity for sst2 (17). The very high affinity of this SS analogue for sst2 probably forms the basis for the higher potency of SOM230 in reducing insulin-like growth factor-I concentrations in rats, primates and dogs (18). Moreover, the very favourable elimination half-life of SOM230, which is 23 h (17), makes this novel compound suitable for clinical application as well.

The present study was carried out to further explore the potential functional significance of sst subtypes expressed in human corticotroph adenomas. We evaluated the effect of the SS analogue SOM230 on ACTH secretion by primary cultures of human corticotroph adenomas and mouse AtT20 corticotroph tumour cells.

Materials and methods

Patients

Pituitary tumour samples were obtained by transsphenoidal operation from 11 patients with Cushing’s disease due a corticotroph adenoma as described in detail previously (19). ACTH-dependent hypercortisolism was biochemically established by the absence of cortisol diurnal rhythm, an increased 24-h free cortisol excretion, insufficient suppression of plasma cortisol concentrations after administration of 1 mg dexamethasone (DEX) and normal to increased plasma ACTH concentrations. Magnetic resonance imaging of the pituitary showed a microadenoma in seven patients, a macroadenoma in one patient and no adenoma in three patients. In these latter patients, pituitary ACTH overproduction was determined by sinus petrosus inferior sampling. Histological examination showed adenomatous tissue (n = 9; case no. 1 and nos 4–11) or hyperplasia (n = 2; case nos 2 and 3) with immunohistochemically expression of ACTH. All patients gave their informed consent for the use of tumour material for research purposes. After surgery, 7 of 11 patients were cured.

In six cases (nos 1–6) the tissue was directly snap-frozen on dry ice and stored at − 80 °C until analysis. Adenoma tissue from the five additional patients (culture nos 7–11) was directly used for cell culturing.

Quantitative PCR

Quantitative PCR was performed as described previously (20). Briefly, poly A+ mRNA was isolated from adenoma tissue using Dynabeads Oligo (dT)25 (Dynal AS, Oslo, Norway). cDNA was synthesized using the poly A+ mRNA, which was eluted from the beads in 40 µl H2O for 2 min at 65 °C, using Oligo (dT)12–18 Primer (Invitrogen). One-twentieth of the cDNA library was used for quantification of sst subtype mRNA levels. A quantitative PCR was performed by TaqMan Gold nuclease assay (Perkin Elmer Corporation, Foster City, CA, USA) and the ABI PRISM 7700 sequence Detection System (Perkin Elmer, Groningen, The Netherlands) for real-time amplifications, according to manufacturer’s protocol. The assay was performed using 15 µl TaqMan Universal PCR Master Mix (Applied Biosystems, Alphen a/d Rijn, The Netherlands), 500 nmol/l forward primer, 500 nmol/l reverse primer, 100 nmol/l probe and 10 µl cDNA template, in a total reaction volume of 25 µl. After an initial heating at 95 °C for 8 min, samples were subjected to 40 cycles of denaturation at 95 °C for 15 s and annealing for 1 min at 60 °C. The primer and probe sequences that were used are indicated below. The detection of hypoxanthine-phosphoribosyl-transferase (HPRT) mRNA served as a control and was used for normalization of the sst subtype mRNA levels. The primer and probe sequences that were used for the detection of sst1, sst2, sst3, sst5 and HPRT mRNAs have been described previously (20). In addition to these primers and probes, we also evaluated sst4 mRNA expression in the present study using the following primers and probe: sst4 forward 5’-CTCCTGGCCCAACCCCTATTCTCTCTC-3’; sst4 reverse 5’-ACCCGCGTGAAAGGATCG-3’; sst4 probe 5’-FAM-TGGCTTCTCTCCGACA-
ACTCCG-TAMRA-3′. Primers and probes were purchased from Biosource (Nivelles, Belgium).

The relative amount of sst subtype mRNA was determined using a standard curve generated from known amounts of human genomic DNA. For determination of the amount of HPRT mRNA, a standard curve was generated of a pool of cDNAs from a human cell line known to express HPRT. The relative amount of sst subtype mRNA was calculated relative to the amount of HPRT mRNA and is given in arbitrary units. Each sample was assayed in duplicate. Poly A+ mRNA from AtT20/D16V cells was isolated as described above. PCR analysis to determine the expression of mouse sst1–5 mRNAs was performed as described elsewhere (21).

Cell dispersion and cell culture

Pituitary adenoma tissue Single cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as described in detail previously (19). For short-term incubation of monolayer cultures, the dissociated cells were plated in 48-well plates (Corning BV Life Sciences, Schiphol-Rijk, The Netherlands) at a density between 10 000 and 50 000 cells per well per 1 ml culture medium. After 3–4 days the medium was changed and 4, 24 or 72 h incubations without or with test substances were initiated. At the end of the incubation the medium was collected and stored at −20°C until determination of ACTH concentrations. For determining the effects of the compounds on cell growth, [3H]thymidine incorporation, as well as the DNA content of the wells, was measured as described in detail elsewhere (23).

Hormone determinations

Human ACTH concentrations were determined by a non-isotopic, automatic chemiluminescence immunnoassay system (Immulite; DPC Inc., Los Angeles, CA, USA). Intra- and interassay coefficient of variation (CV) values were 5.6 and 7.8% respectively. Human growth hormone (GH), prolactin (PRL), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations in the media were determined, in order to exclude the presence of contaminating normal pituitary cells in the cultures. GH, PRL, LH and FSH concentrations were determined by a non-isotopic, automatic chemiluminescence immunoassay system as well (Immulite; DPC). Intra- and interassay CV values for GH, PRL, LH and FSH were 6.0, 5.7, 5.7 and 6.4% and 6.2, 6.4, 12.3 and 7.5% respectively. Except for the expected hormone ACTH, none of the other hormones was detected (not shown).

Test substances

OCT (Sandostatin) was obtained from Novartis Pharma AG (Basle, Switzerland). SOM230 was a gift from Novartis Pharma AG. SS-14 was purchased from Sigma Chemical Co. Binding affinities of SS-14, OCT and SOM230 to the five sst subtypes are shown in Table 1. DEX was obtained from the Erasmus MC Pharmacy (Rotterdam, The Netherlands). CRH was purchased from Ferring (Hoofddorp, The Netherlands).

Statistical analysis of the data

All data on hormone release are expressed as means±s.e., n = 4 wells per treatment group. All data were analysed by ANOVA to determine overall differences between treatment groups. When significant differences were found by ANOVA, a multiple comparison between treatment groups was made using the Newman–Keuls test. Calculation of IC50 values for inhibition of hormone release were made using GraphPad Prism (San Diego, CA, USA).
Table 1 Binding affinity of SS-14, OCT and SOM230 for the five human sst subtypes, sst1–5. Data are reproduced with permission from the society of the European Journal of Endocrinology (17). Results are mean±S.E. IC_{50} values (nmol/l).

<table>
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<th>Compound</th>
<th>hsst1</th>
<th>hsst2</th>
<th>hsst3</th>
<th>hsst4</th>
<th>hsst5</th>
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<td>SS-14</td>
<td>0.93±0.12</td>
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<td>OCT</td>
<td>228±80</td>
<td>0.38±0.08</td>
<td>7.1±1.4</td>
<td>&gt;1000</td>
<td>6.3±1.0</td>
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<tr>
<td>SOM230</td>
<td>9.3±0.1</td>
<td>1.0±0.1</td>
<td>1.5±0.3</td>
<td>&gt;100</td>
<td>0.16±0.01</td>
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**Results**

**sst subtype mRNA expression**

In six out of six human corticotroph adenomas (nos 1–6), sst2 mRNA was detectable. However, the relative copy numbers were low (varying between 8 and 141 copies/HPRT). The relative sst2 copy number was considerably lower compared with the majority of GH-secreting pituitary adenomas, as recently reported (20). sst3 mRNA was detectable at much higher levels in six out of six adenomas (relative copy number between 277 and 1217). In the two cases in which histological analysis of the tissue revealed hyperplasia of ACTH-producing cells (case nos 2 and 3), sst5 mRNA levels were relatively high. On the other hand, one ACTH-secreting pituitary adenoma (case no. 1) expressed the highest level of sst5 mRNA in the series. sst1, sst3 and sst4 mRNA was detectable at low levels in only one, two and two out of six samples respectively (Fig. 1).

In agreement with the results of RT-PCR analysis of the corticotroph adenomas, the sst subtype mRNAs expressed in ACTH-secreting mouse AtT20 corticotroph pituitary tumour cells were sst2, sst3 (low abundance) and sst5 (Fig. 2).

**Effect of SS-14, OCT and SOM230 on basal and CRH-induced ACTH secretion by primary corticotroph adenoma cells**

Since not sufficient tissue was obtained to carry out both mRNA and culture studies on the same tissue, cell culture experiments were performed using adenoma tissue from five additional patients (nos 7–11). In a 72 h incubation, SOM230 (10 nmol/l) significantly suppressed ACTH secretion in three out of five primary cultures of human corticotroph adenomas (between 30 and 40% suppression). In contrast, OCT (10 nmol/l) suppressed basal ACTH release in only one out of five cultures (28% suppression) (Fig. 3A and B). From one corticotroph adenoma (no. 8) sufficient cells were obtained to perform a time-course study of the effects of SS-14, OCT and SOM230 on basal ACTH release. As shown in Fig. 4A (4, 24 and 72 h incubation), statistically significant (P < 0.01 vs untreated control cells) suppression of ACTH release was observed only after 72 h of incubation with SOM230 (10 nmol/l) and SS-14 (10 nmol/l). Ten nmol/l of OCT did not significantly inhibit basal ACTH release at any time-point. A comparable time-course experiment was performed in mouse AtT20 cells. In agreement with the observations in primary human corticotroph adenoma cultures, SOM230 and SS-14, but not OCT, all tested at 10 nmol/l, suppressed basal ACTH production (Fig. 4B). The effect of SOM230 on basal ACTH release by AtT20 cells was dose-dependent with an IC_{50} value of 0.2 nmol/l (Fig. 5A), corresponding to the binding affinity of SOM230 for sst5. In contrast, OCT inhibited ACTH release with much lower potency with a slight suppression at 100 nmol/l (Fig. 5A). Taken together, these data suggest that the effect of SOM230 on basal ACTH release is most probably mediated via sst5. OCT and SOM230 did not inhibit AtT20 cell proliferation as measured by [3H]thymidine incorporation (Fig. 5B), and had no effect on the DNA content of the cells (not shown).

In contrast to the absence of an effect of OCT on basal ACTH release by AtT20 cells, OCT (10 nmol/l) significantly suppressed CRH (10 nmol/l)-induced ACTH release by 52% and SS-14 by 47% in a 3 h incubation. SOM230 was significantly more potent and inhibited CRH-induced ACTH release by 76% (Fig. 6). The effect of SS-14 on CRH-induced ACTH-release by AtT20 cells is in agreement with previous reports (24–26).

**Effect of glucocorticoid pre-treatment**

In order to evaluate whether pre-treatment of the cells with glucocorticoids influenced the sensitivity to the effects of OCT and SOM230, mouse AtT20 cells were pre-treated with 10 nmol/l DEX during 48 h, after which a 3 h incubation without or with CRH (10 nmol/l), OCT (1 nmol/l) and/or SOM230 (1 nmol/l) was performed. Figure 7 shows that pre-treatment of AtT20 cells with 10 nmol/l DEX had no effect on the inhibitory effect of SOM230 on CRH-induced ACTH release. On the other hand, the inhibitory effect of 1 nmol/l OCT was completely abolished by DEX pre-treatment. In the presence of DEX, SOM230 almost completely abolished CRH-induced ACTH release (Fig. 7, lower panel). Finally, DEX pre-treatment did not change the inhibitory effects of 10 nmol/l OCT or SOM230 on CRH-induced ACTH release (data not shown), suggesting that OCT at 10 nmol/l induced its inhibitory effect via interaction with sst5, for which it has significantly lower affinity...
compared with SOM230 (Table 1). Moreover, in one primary culture of corticotroph adenoma cells (case no. 4), which was unresponsive to OCT, DEX (10 nmol/l) pre-treatment did not reduce the inhibitory effect by SOM230 on CRH (10 nmol/l)-induced ACTH release as well (21% without DEX vs 33% in the presence of DEX).

Discussion

Currently, there is no effective medical treatment option in patients with pituitary-dependent Cushing’s disease (3, 4). Therefore, in the present study we evaluated the effects of the recently developed multi-ligand stable SS analogue SOM230 (17, 18, 27), which has high binding affinity to sst1, sst2, sst3 and sst5, on ACTH release by human corticotroph tumours in vitro. The role of SS in the regulation of normal ACTH release is equivocal. Previous in vitro studies showed no inhibitory effect of SS on basal and CRH-induced ACTH release by normal rat anterior pituitary cells (28) and perfused normal rat pituitary halves (29). On the other hand, SS inhibited CRH-stimulated ACTH release by fragments of pituitary glands (30), as well as arginine vasopressin-induced ACTH release by cultured pituitary cells from long-term adrenalectomized rats (31). The latter study suggests that normal corticotrophs only respond to SS in the presence of very low cortisol concentrations. Confirming this hypothesis, we demonstrated that SS inhibits CRH-stimulated ACTH secretion by normal rat anterior pituitary cells only when the cells are cultured in the absence of glucocorticoids in the culture medium (32). In addition, pre-incubation of the cells with the...
progesterone-glucocorticoid receptor-blocking compound RU 38 486, increased the sensitivity of ACTH secretion to the inhibitory effect by SS and pre-treatment with DEX made the cells insensitive to SS (32). The high levels of cortisol in patients with pituitary-dependent Cushing’s disease can thus be responsible for the observed lack of inhibition of ACTH release by SS and/or OCT in these patients (6, 11, 33). Moreover, in patients with Nelson syndrome and adrenal insufficiency of different origins, SS and/or OCT lower ACTH secretion (6, 9, 34, 35). In cultured corticotroph adenomas, SS and/or OCT inhibit ACTH secretion in part of the cultures (11, 36–38). Stalla et al. (11) showed that hydrocortisone treatment in vitro abolished the inhibitory effect of OCT on ACTH secretion, possibly due to a downregulation of sst on the corticotrophs.

In agreement with this hypothesis, cortisol reduced $[^{125}\text{I}]-\text{Tyr}^1$ SS binding on mouse AtT20/D16 pituitary tumour cells by 20–40% (39), although the sst subtype was not characterized.

Little is known with respect to sst subtype expression in pituitary corticotrophs. In rat pituitary cells, co-localization of all five sst subtypes with ACTH-expressing cells has been reported (40). In another study, sst$_5$ mRNA was found in 38% of normal corticotrophs, and sst$_2$ in only 3%. Moreover, the average number of grains per cell was also higher for sst$_5$ than sst$_2$ (12). This suggests that under normal physiological conditions, sst$_5$ is predominantly more expressed in rat corticotrophs than sst$_2$. As far as is known, no data on the quantitative expression of sst mRNA in human corticotroph adenomas are available. In the present study we found that at the mRNA level, sst$_5$ was the predominantly expressed receptor type in human corticotroph adenomas. In the small series of tissues ($n = 6$) analysed in this study, two cases of hyperplasia of corticotroph cells expressed a relatively high sst$_5$ mRNA level. On the other hand, one corticotroph adenoma expressed the highest sst$_5$ mRNA level in this series. The number of cases is too low, however, to establish whether there is a difference in sst$_5$ mRNA expression between hyperplasia of ACTH-producing cells and ACTH-secreting adenoma. sst$_2$ mRNA was expressed in all cases as well, although at a much lower level. The high frequency of expression of sst$_2$ and sst$_5$ in corticotroph adenomas is well in agreement with other reports. mRNA expression in human corticotroph tumours shows the overall presence of sst$_1$ in five out of eight (63%), sst$_2$ in seven out of eight (88%), sst$_3$ in three out of nine (33%), sst$_4$ in one out of eight (12%), and sst$_5$ in six out of seven (86%) cases (13–15, 41, 42). The low expression levels of sst$_2$ may explain the lack of efficacy of the sst$_2$-preferring agonist OCT in lowering circulating ACTH and cortisol levels in untreated patients with pituitary-dependent Cushing’s disease (6, 11). In vitro we found a statistically significant inhibition of basal ACTH release by OCT in only one out of five cases. The higher number of cultures (three out of five) responding to a maximally active concentration of 10 nmol/l SOM230, which has very high binding affinity for sst$_5$ mRNA. Unfortunately, not enough tissue was obtained to perform both mRNA analysis and cell culture studies. In one adenoma, which had an intermediate sst$_5$ mRNA level (case no. 4), SOM230 already inhibited ACTH secretion, whereas OCT had no effect. It should be noted that only mRNA levels were studied. Future studies should demonstrate whether sst$_5$ receptors are expressed in corticotroph adenomas at a high protein level as well. The importance of sst$_5$ in regulating ACTH release was further confirmed by our observation that SOM230 was also much more potent compared with OCT in inhibiting basal ACTH release by mouse AtT20/D16V corticotroph tumour cells. The pattern of inhibition, e.g. a higher potency of SOM230 vs OCT, is

Figure 3 Effect of 10 nmol/l OCT (A) and 10 nmol/l SOM230 (B) on basal ACTH release from five cultured human corticotroph adenomas (nos 7–11). The cells were incubated in the absence or presence of OCT (A) or SOM230 (B) during 72 h after which the medium was collected for ACTH determination. Values are expressed as per cent of control (CON; untreated cells). Basal ACTH concentrations in the culture media were 239±5.0, 28 800±1159, 25 100±2150, 218±12 and 3228±153 fmol/well, for cultured adenomas nos 7, 8, 9, 10 and 11 respectively. *$P < 0.05$ and **$P < 0.01$ vs control. Means±S.E., $n = 4$ wells per treatment group.

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in line with the sst binding profile of both SS analogues. Recently, Cervia et al. (43) showed that sst2 is the predominant functional receptor subtype in AtT20 cells, while sst5 is also able to mediate inhibition of ACTH release when the ligand is not able to activate sst2 receptors. Interestingly, while SOM230 was much more potent in inhibiting basal ACTH release by AtT20 cells, we found that OCT also potently inhibited CRH-induced ACTH release. However, maximal inhibition of ACTH by SOM230 was significantly higher compared with OCT. These data suggest that expression of sst2 in our AtT20 line is relatively low, compared with sst5. The involvement of both sst2 and sst5 in the regulation of ACTH release is further underlined by the observation that sst2- and sst5-specific agonists potently inhibit ACTH release and cAMP production by AtT20 cells (44).

As indicated above, glucocorticoids may downregulate sst on AtT20 corticotrophs and lower responsiveness of ACTH to the inhibitory effect of SS and/or OCT. Therefore, we also studied whether pre-treatment of the cells with the synthetic glucocorticoid DEX (10 nmol/l) reduced responsiveness of the cells to inhibition of ACTH release by SOM230. We found that glucocorticoid pre-treatment did not influence the inhibitory effect of SOM230 on CRH-induced ACTH release, suggesting that the expression of functional sst5 receptors is relatively resistant to glucocorticoids. This may also explain the higher expression levels of sst5 mRNA we found in human corticotroph adenomas from untreated patients with pituitary-dependent Cushing’s disease. These data suggest that glucocorticoids may have differential regulatory effects on sst2 and sst5 expression. An intriguing observation is that SOM230 and SS-14 only inhibit basal ACTH secretion after prolonged in vitro exposure. SOM230 did not inhibit AtT20 cell proliferation. Therefore, other mechanisms, e.g. inhibition of pro-opiomelanocortin synthesis and/or increased ACTH breakdown, as has been shown for the effect of OCT on GH-secreting pituitary adenomas (45, 46), may form additional explanations for the inhibitory effect of SOM230 and SS-14 on basal ACTH secretion. Interestingly, recent evidence suggests that sst5 is also important in the control of ACTH secretion in vivo. sst5 knock-out mice were shown to have significantly elevated ACTH and corticosterone levels, compared with wild-type mice (47).

While our data suggest that the novel SS analogue SOM230 may be useful in the medical management of

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**Figure 4** Time-dependent effect of OCT (10 nmol/l), SOM230 (10 nmol/l) and SS-14 (10 nmol/l) on basal ACTH release from cultured human corticotroph adenoma no. 8 (A) and mouse AtT20 corticotroph tumour (B) cells. The cells were incubated for 4, 24 or 72 h in the absence or presence of the indicated compounds, after which medium was collected for ACTH determination. Values are expressed as the per cent of ACTH release by control (untreated) cells at the indicated time-points. For the human corticotroph adenoma cells, basal ACTH values were 3505±232 (4 h), 9778±946 (24 h) and 28 800±1159 (72 h) fmol/well. *P < 0.01 vs control. Means±S.E., n = 4 wells per treatment group.

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patients with pituitary-dependent Cushing’s disease, several issues will have to be clarified. First, prolonged treatment of AtT20 cells with SS-14 results in desensitization of its inhibitory effect on ACTH secretion and cAMP formation (48, 49). In addition, prolonged agonist exposure of AtT20 cells with SS-14 or SS-28 was shown to downregulate SS-14 receptors (50). However, these studies only evaluated the acute inhibitory effects of SS-14 on AtT20 cells. Moreover, the effects of SOM230 are most likely sst5-mediated and sst5 receptors have shown to be rapidly recycled and recruited from intracellular storages after agonist activation. This combination of recycling and recruitment of spare sst5 receptors may protect from long-term downregulation through sequestration and, therefore, facilitate extended SS signalling (51). We observed that the inhibitory effects of SOM230 on basal ACTH secretion became only apparent after prolonged exposure. This observation already suggests that endogenously expressed sst5 receptors may be more resistant to desensitization and/or downregulation. Nevertheless, the in vivo effect of SOM230 on ACTH secretion by corticotroph adenomas needs to be further evaluated.

In conclusion, the selective expression of sst5 receptors in human corticotroph adenomas, in combination with the inhibitory effect of SOM230 on basal and CRH-induced ACTH secretion, suggests that SOM230 may have potential in the medical treatment of pituitary-dependent Cushing’s disease.
Effect of glucocorticoids on OCT- and SOM230-mediated inhibition of CRH-stimulated ACTH release by mouse AIT20 pituitary adenoma cells. AIT20 cells were pre-incubated during 48 h without or with 10 nmol/l DEX. After 48 h, the medium was refreshed and the cells were incubated for 3 h in the absence or presence of DEX, CRH (10 nmol/l) and OCT (1 nmol/l) or SOM230 (1 nmol/l), after which the medium was collected for ACTH determination. *P < 0.01 vs control, #P < 0.01 vs CRH alone. Means ± s.e., n = 4 wells per treatment group.

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Received 30 September 2004
Accepted 14 December 2004

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