Regulation of CRH-induced secretion of ACTH and corticosterone by SOM230 in rats

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Abstract

Objective: Adrenocorticotropic hormone (ACTH)-dependent Cushing’s syndrome is biochemically characterized by increased plasma concentrations of ACTH inducing hypersecretion of cortisol. Somatostatin is known to inhibit ACTH secretion, and in vitro data have shown the inhibition of ACTH secretion by agonists activating sst2 and sst5 receptors. The present study aimed to determine the inhibitory effect of the multireceptor ligand SOM230, compared with the sst2-prefering agonist octreotide, on corticotropin-releasing hormone (CRH)-stimulated secretion of ACTH and corticosterone in rats.

Methods: Secretion of ACTH and corticosterone was induced by i.v. application of CRH (0.5 μg/kg) in rats pretreated 1 h before by i.v. application of SOM230 (1, 3, or 10 μg/kg), octreotide (10 μg/kg) or NaCl 0.9%.

Results: SOM230 (3 and 10 μg/kg) inhibited CRH-induced ACTH release by 45±3% and 51±2%, respectively, and corticosterone release by 43±5% and 27±16%, respectively. 10 μg/kg of octreotide tended to be less potent at inhibiting ACTH release (34±6% inhibition) and did not alter the secretion of corticosterone.

Conclusion: SOM230 has a stronger inhibitory effect on ACTH and corticosterone secretion than octreotide in rats. This difference can be explained by its higher affinity to sst1, sst3 and especially sst5 receptors compared with octreotide.

Introduction

Cushing’s syndrome is characterized by centripetal obesity, skin manifestations, hypertension, proximal myopathy, insulin resistance, hyperlipidemia, osteopenia and some psychiatric manifestations. Adrenocorticotropic hormone (ACTH)-dependent forms of the syndrome include Cushing’s disease, ectopic ACTH-producing tumors, and corticotropin-releasing hormone (CRH)-producing tumors. In these three cases, increased plasma concentrations of ACTH induce hypersecretion of cortisol and a bilateral adrenocortical hyperplasia (1). ACTH secretion is physiologically inhibited by somatostatin (2), a 14 or 28 amino acid cyclopeptide involved in the regulation of hormone and growth factor secretion (3). Somatostatin effects are mediated through five G protein-coupled receptors, sst1–sst5, and can be mimicked by analogs which have different affinities for the five sst receptors. Among them are the sst2-prefering agonist octreotide, and the multireceptor ligand SOM230 which binds to sst1, sst2, sst3 and sst5 with high affinity, having a 30, 5 and 40 fold higher affinity to sst1, sst3 and sst5 receptors, respectively, compared with octreotide (4). It has been reported that sst2- and sst5-specific agonists inhibit CRH-induced ACTH release from AtT-20 cells (5). Although individual clinical case reports indicate that octreotide was able to restore eucortisolemia in patients presenting with an ACTH-dependent Cushing’s syndrome (6), the efficacy of the sst2-prefering agonist, octreotide, could not be established in clinical trials (7). Most recently, Hofland et al. showed the predominant expression of sst5 receptors in cultured human corticotroph adenomas (8). In this study, the secretion of ACTH from cultured human adenomas was inhibited by SOM230. These in vitro data strongly argue for the usefulness of somatostatin analogs with high affinity for sst2 and sst5 receptors, such as SOM230, to regulate ACTH plasma levels in Cushing’s patients. However, the inhibition of ACTH secretion by somatostatin analogs in vivo has not yet been demonstrated. Therefore, the aim of the present study was to investigate the effect of SOM230 on the stimulated secretion of ACTH and corticosterone, the rodent equivalent of cortisol, in a freely moving rat model.

Materials and methods

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

**Compounds and formulations**

SOM230 acetate was dissolved in sterile water, and stock solutions stored frozen at −20 °C. On the day of experiment, the stock solutions were diluted with 0.9% sterile saline to the final concentrations as indicated (pH 5–6). SMS 201-995 acetate (octreotide) was prepared in the same way as SOM230. CRH (human/rat, Bachem AG, Bubendorf, Switzerland) was dissolved in water, and stocks stored frozen at −20 °C.

**Animals and treatments**

Adult male Sprague-Dawley rats (Ico:OF A-SD, Iffa-Credo, Lyon, France) were housed in groups of five rats per cage under controlled light (12 h light:12 h dark) and temperature (22 °C). The rats were fed with water and laboratory food *ad libitum*. Animals were weighed on the day of surgery. The rats were allowed to adjust to the light/dark cycle for 1 week prior to the experiment. They were used with a body weight of 275–336 g. On the day before the experiment (day 0), rats were anesthetized by inhalation of isoflurane 5% in oxygen/air and kept under anesthesia with isoflurane 2% in oxygen/air. The right femoral vein and artery were catheterized in order to perform i.v. injections and blood sampling, respectively. After surgery, rats were placed individually in cages, and the arterial catheter was connected to a pump with constant infusion of NaCl 0.9% containing heparin 10 IU/ml. On day 1, animals were pretreated i.v. with either SOM230 (1, 3 or 10 μg/kg), octreotide (10 μg/kg) or saline (NaCl 0.9%) for 1 h. Then they were injected i.v. with CRH 0.5 μg/kg to stimulate the secretion of ACTH and corticosterone. The injection of CRH was considered as the time point 0 min. Blood samples were collected at time points (—60, 0, 10, 15, 20, 120 min, in pre-chilled Eppendorf tubes containing EDTA (WED-19, Milian SA, Geneva, Switzerland) and aprotinin (100 U/ml, Calbiochem, Merck KGaA, Darmstadt, Germany). The plasma was separated by centrifugation and frozen until measurement of hormone levels. Catheterized, non-anaesthetized rats were chosen in order to avoid the effect of acute stress on ACTH levels caused by handling and blood sampling. Also, the experiment was performed one day after surgery to allow the rats to recover from surgery, and to prevent any influence of the anesthetics used, i.e. isoflurane.

**Analytical methodology**

Plasma concentrations of ACTH and corticosterone were determined according to the manufacturer’s instructions, by radioimmunoassay (RIA) using commercially available kits: (125I) ACTH RIA kit ( Diasorin, Stillwater, Minnesota); (125I)corticosterone RIA (ICN Biomedicals GmbH, Eschwege, Germany).

**Statistical analysis**

Results are expressed as mean±S.E.M. Data obtained at each time point were compared between groups using the non-parametric Kruskal–Wallis test and a Dunn’s multiple comparison test, with the program GraphPad Prism, version 4.02.

**Results**

The basal plasma ACTH concentration in rats was 46±3 pg/ml (mean±S.E.M., n = 6). After i.v. injection of CRH (0.5 μg/kg), ACTH levels increased up to 229±52 pg/ml within 20 min (Fig. 1A). Similarly, corticosterone levels increased from 60±26 to 374±24 ng/ml (Fig. 1B). Before stimulation with

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Figure 1 CRH-induced increase in rat plasma ACTH (A) and corticosterone (B). Rats were pretreated 1 h before with SOM230 1 μg/kg ●, 3 μg/kg ▲, or 10 μg/kg ● (red), octreotide 10 μg/kg ● (blue) or NaCl 0.9% ■. ACTH release was stimulated by i.v. injection of CRH (0.5 μg/kg). Blood samples were taken at time points —60, 0, 10, 15, 20 and 120 min. ACTH and corticosterone concentrations were determined by RIA in rat plasma. Results are expressed as mean±S.E.M. of data from seven rats for SOM230 and octreotide, and eight rats for vehicle group. * P < 0.05, ** P < 0.01 vs (NaCl + CRH) group.

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CRH, rats were pretreated 1 h before with either SOM230 (1, 3, or 10 μg/kg, i.v.), octreotide (10 μg/kg, i.v.) or saline. SOM230 at 1 μg/kg did not alter CRH-induced ACTH and corticosterone secretion. Pretreatment of rats with SOM230 at 3 and 10 μg/kg resulted in a 56±12% and a 70±5% (P = 0.011) decrease in plasma ACTH levels, respectively, compared with saline-treated control animals at time point 20 min (Fig. 1A). Octreotide at 10 μg/kg also inhibited CRH-induced ACTH release (55±6% decrease), but tended to be less potent than SOM230, although the difference between both compounds was not significant. Accordingly, the calculation of the area under the curve (AUC) from time—60 min to time 120 min showed a dose-dependent inhibition of CRH-induced ACTH secretion by SOM230, with 45±3% inhibition at 3 μg/kg and a 51±2% inhibition at 10 μg/kg (P = 0.015). Octreotide at 10 μg/kg did not significantly inhibit ACTH secretion (34±6% decrease, P > 0.05). SOM230 also inhibited CRH-induced corticosterone secretion (Fig. 1B). At 3 μg/kg SOM230, the corticosterone levels were reduced by 43±5% (P = 0.069 for the Kruskal–Wallis test, P < 0.05 for Dunn’s multiple comparison test), and at 10 μg/kg SOM230 the corticosterone levels were reduced by 27±16%, as calculated from the AUC values. However, the reduction did not reach statistical significance (Table 1). Octreotide did not alter CRH-induced corticosterone secretion (Fig. 1B, Table 1).

Discussion

This study demonstrates the inhibition of CRH-induced secretion of both ACTH and corticosterone by the multi-receptor ligand SOM230 in vivo in rats. Octreotide did not inhibit CRH-induced corticosterone secretion, and tended to be less efficacious than SOM230 for the inhibition of ACTH release. The effect observed for SOM230 on ACTH secretion was expected since it had previously been described that sst2- and sst5-specific agonists inhibit CRH-induced ACTH release from the mouse pituitary cell line AtT-20 (5). In addition, sst receptor expression studies on pituitary gland tissue and purified pituitary cells showed the expression of both sst2 and sst5 in corticotrophs (9, 10). Most recently, an elegant in vitro study by Holland et al. has shown that SOM230 inhibits ACTH secretion from primary cultures of human corticotroph adenomas, whereas octreotide was not able to inhibit ACTH secretion in most cases (8). The difference in activity of the two somatostatin analogs was associated with a predominant expression of sst5 receptor mRNA in these cells. The stronger effect of SOM230 compared with octreotide observed in the present study may be explained by the binding properties of the two compounds to sst receptors. Indeed, SOM230 binds with sub-nanomolar affinity to sst2 and sst5 receptors (IC50 values of 1±0.1 nM and 0.16±0.01 nM, respectively (4)), whereas octreotide has a preferential affinity for sst2 receptors compared with sst5 (IC50 values of 0.38±0.08 nM compared with 6.3±1 nM (4)). The differences are even more pronounced with regard to their functional activity, as reported for the inhibition of forskolin-induced intracellular cAMP accumulation (11). Clinical studies have shown that in most cases, octreotide fails to normalize ACTH and cortisol levels in Cushing’s disease patients (7). However, octreotide could reduce ACTH plasma levels in patients with Nelson’s syndrome (12). This difference of efficacy may be explained by a receptor subtype specific downregulation of sst receptors by the high cortisol plasma levels observed in Cushings’ patients, as recently suggested (8). According to this hypothesis, recent in vitro studies have shown that the cortisol analog dexamethasone was able to abolish the effect of octreotide on ACTH secretion, but did not affect the inhibition produced by SOM230. This difference was associated with a down-regulation of sst2 and no change in sst5 receptor expression (8, 13). In the present study, CRH-induced corticosterone increase was inhibited by SOM230, but not by octreotide. This may suggest a direct effect of SOM230 on adrenals through a receptor other than sst2. Indeed, the expression of sst receptors has been reported in adrenal tumors (14) and in the rat adrenal gland (15). mRNA for sst1, sst2, sst4 and sst5 was detected by RT-PCR with a predominant amount of sst2, and in situ hybridization showed major localization of sst2 receptor mRNA in the zona glomerulosa, but also some labeling in the external

**Table 1** Area under curve (AUC) calculated from the data represented in Fig. 1. Results are expressed as mean±S.E.M., n = 7 for SOM230 and octreotide, and n=8 for vehicle.

<table>
<thead>
<tr>
<th>Group</th>
<th>ACTH (ng/min/ml⁻¹)</th>
<th>Corticosterone (μg/min/ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 0.9% + CRH 0.5 μg/kg</td>
<td>20.18±3.20</td>
<td>36.87±4.37</td>
</tr>
<tr>
<td>SOM230 1 μg/kg + CRH 0.5 μg/kg</td>
<td>23.23±7.23</td>
<td>30.41±4.21</td>
</tr>
<tr>
<td>SOM230 3 μg/kg + CRH 0.5 μg/kg</td>
<td>11.00±1.46*</td>
<td>21.14±1.80*</td>
</tr>
<tr>
<td>SOM230 10 μg/kg + CRH 0.5 μg/kg</td>
<td>9.86±1.03*</td>
<td>26.85±5.68</td>
</tr>
<tr>
<td>Octreotide 10 μg/kg + CRH 0.5 μg/kg</td>
<td>13.24±2.89</td>
<td>33.56±5.62</td>
</tr>
</tbody>
</table>

* P < 0.05 vs (NaCl+CRH) group.
part of zona fasciculata (15). Therefore, a direct effect of somatostatin analogs on corticosterone secretion cannot be excluded at present. However, the fact that SOM230 has a relatively stronger inhibitory effect on CRH-stimulated ACTH secretion than on corticosterone secretion is in line with a primary action on the pituitary. These are the first preclinical data comparing the effects of SOM230 and octreotide on ACTH secretion in vivo. The present study, together with the in vitro data reported by others (8) support the idea that SOM230 may have potential for the regulation of ACTH plasma levels. This would be of major interest for the treatment of ACTH-dependent Cushing’s syndrome, which includes Cushing’s disease, ectopic ACTH-producing tumors, and CRH-producing tumors, as well as Nelson’s syndrome, where the elevated secretion of ACTH needs to be controlled. Further studies in animal models showing increased or decreased levels of ACTH and/or corticosterone might help to further strengthen this concept.

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References


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