Somatostatin Analogue (SMS 201-995) Decreases Plasma Levels of Corticotropin (ACTH) and Corticotropin-Releasing Hormone in a Patient with Ectopic ACTH-Producing Tumors

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Abstract

Effects of long-acting somatostatin analogue (SMS 201–995) on plasma corticotropin (ACTH) and corticotropin-releasing hormone (CRH) levels were studied in a patient (63-year-old woman) with ectopic ACTH-producing tumors associated with type I multiple endocrine neoplasia (MEN-I). The patient had undergone bilateral adrenalectomy. Plasma CRH, as well as plasma ACTH, beta-endorphin and alpha-MSH, increased. The hormone levels were dramatically decreased by acute administration of SMS 201–995. Moderately higher doses of dexamethasone (0.05 or 0.1 mg/kg a day) did not decrease plasma CRH or ACTH. An extremely high dose of dexamethasone (0.2 mg/kg a day), however, decreased plasma ACTH, but failed to decrease plasma CRH. Acute administration of SMS 201–995 further lowered the level of plasma ACTH even in this condition. In addition to the decrease in ACTH, SMS 201–995 decreased plasma CRH. Chronic administration of SMS 201–995 continuously decreased plasma CRH, ACTH and beta-endorphin. The decrease in these hormone concentrations accompanied the disappearance of hyperpigmentation.

These results suggested that SMS 201–995 inhibits hypersecretion not only of ACTH but also of CRH, and that the agent is therapeutically useful in normalizing the hypersecretion of these hormones.

Somatostatin (SRIF) or long-acting SRIF analogue (SMS 201–995) has been reported to be effective in suppressing the secretion of pituitary hormones, such as growth hormone (GH) in patients with acromegaly (Yen et al., 1974, Plewe et al., 1984, Kelijman et al., 1988), thyrotropin (TSH) in patients with TSH-secreting pituitary tumors (Comi et al., 1987, Wemeau et al., 1988) and corticotropin (ACTH) in patients with Nelson’s syndrome (Tyrell et al., 1975, Benker et al., 1976, Lamberts et al., 1989)

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or with ectopic ACTH secretion (Bertagna et al., 1989). However, it is not certain whether the agent is effective in inhibiting the release of hypothalamic releasing factors, such as GH-releasing hormone (GH-RH), TSH-releasing hormone (TRH) and ACTH-releasing hormone (CRH) (Lamberts, 1988).

We examined a patient with multiple endocrine neoplasia type I (MEN-I), who had ectopic (metastatic) ACTH-producing tumors with a high level of plasma CRH. In order to inhibit the hypersecretion of ACTH, SMS 201–995 was administered. During the administration, plasma ACTH and CRH levels were monitored, and we obtained results which suggested that the agent could suppress the secretion not only of ACTH but also of CRH. In this report, we present the case and discuss the mechanism of SRIF inhibition of ACTH and CRH release in this patient.

**Materials and Methods and Measurement of Hormones**

The serum levels of gastrin, insulin, calcitonin, prolactin (PRL), 3, 5, 3'-triiodo-L-thyronine (T₃), L-thyroxine (T₄), thyroid stimulating hormone (TSH) (Daiichi Radioisotope, Inc., Tokyo, Japan), growth hormone (GH) (Dainabot Inc., Tokyo, Japan), and parathyroid hormone (PTH) (Yamas Shouyu Co., Ltd., Tokyo, Japan) were measured by RIAs with commercially available kits as previously described (Hiramatsu et al., 1983). Vasoactive intestinal peptide (VIP), pancreatic polypeptide (PP) (Mayo Medical Laboratory Co., New York, U. S. A.), insulin-like growth factor I (IGF-I) (Eiken Immunochemical Co., Tokyo, Japan) were also measured by RIAs with commercially available kits. Plasma levels of beta-endorphin, SRIF, glucagon (Special Reference Laboratory Inc., Tokyo, Japan), and alpha-MSH (Dainabot Inc.) were measured by RIAs with commercially available kits. Plasma ACTH was also measured by RIA with a commercially available kit (Diagnostic products Co., Los Angles, U. S. A).

The inter- and intra-assay coefficients of variation were 5.4 and 6.6% respectively for ACTH concentrations ranging 10–200 pg/ml. The levels of ACTH were confirmed by using another RIA kit (Compagnie Oris Industrie S. A. France), in which the interassay coefficient of variation was 6.1% for ACTH concentrations ranging 5–300 pg/ml. CRH extracted from serum by affinity column chromatography was measured by RIA according to the method of Suda et al. (1985). The inter- and intra-assay coefficients of variation were 6.2 and 6.7% respectively for CRH concentrations ranging 2–200 pg/ml. Anti-CRH antibody used in RIA detected CRH which was exogenously applied to HPLC (high performance liquid chromatography). The elution position of plasma CRH (a single peak) in HPLC corresponded to the position of CRH exogenously applied. The sensitivity of the CRH assay was 2.0 pg/ml. All samples of CRH were measured in the same assay. The plasma concentration of dexamethasone was measured by HPLC.

The immunocytochemical localization of ACTH, beta-endorphin, beta-MSH or CRH was accomplished by using an avidin biotinylated-peroxidase complex (ABC) procedure (Vector Kits, Burlingame, California, U. S. A.) (Hsu et al., 1981) according to the method of Lloyd et al. (1987). The immunohistochemistry was carried out on routinely formalin-fixed parafin sections of the tissues by using rabbit anti-ACTH (1:500), purchased from Immuno Nuclear, Stillwater (U. S. A.), anti-beta-endorphin (1:80), donated by Dr. K. Abe, National Cancer Center, Tokyo, Japan, anti-beta-MSH (1:500), purchased from Im UCB-Bioproducts (U. S. A.), and anti-CRH (1:500), kindly provided from Dr. A. V. Shally, Veterans Administration Medical Center, Tulane University School of Medicine, for primary antibodies. Nuclear counter-staining was done with methyl green. The specificity of staining was confirmed by absorption tests, using respective synthetic antigens (0.1 μg/μl of each diluted antiserum). SMS 201–995 (Sandostatin) was purchased from Sandoz Ltd. (Basel, Switzerland).

**Case Presentation**

A 63-year-old woman (height 152.0 cm and body weight 51.0 kg) was admitted to
our hospital with pigmentation of the lips and tongue. A diagnosis of multiple endocrine neoplasia type I (MEN-I) had been made three years before the admission. The patient had been operated (total thyroparathyroidectomy with auto-transplantation of a part of the parathyroid gland to the forearm, pancreateo-duodenectomy with Whipple's procedure, and bilateral adrenalectomy) because of primary hyperparathyroidism (4 parathyroid glands hyperplasia), adenomatous goiter, pancreatic (pancreatic head) islet tumor (endocrine function had not been evaluated), and hypertrrophy of bilateral adrenal glands associated with Cushing's syndrome. At that time, neither enlargement of the pituitary gland nor tumor of pituitary had been detected by computed tomography (CT). Further, ectopic ACTH secretion had not been identified at that time. After the operations, hydrocortisone (0.5 mg/kg a day), T4 (2 µg/kg a day) and 1-alpha-hydroxy vitamin D₃ (2.0 µg/day) had been administered.

After admission, the serum and plasma concentrations of hormones were measured. GH and PRL responses to TRH were normal. Serum gastrin, calcitonin and PTH were normal. Serum insulin had increased slightly. Serum TSH and thyroid hormones (T₃ and T₄) had been maintained in the normal range by supplementing with T₄ (2 µg/kg a day). Glucagon, IGF-I, VIP, PP and SRIF in plasma were all normal. Table 1 summarizes the levels of the hormones measured.

Plasma levels of ACTH, alpha-MSH, beta-endorphin, and CRH were extremely high even when the patient was given hydrocortisone (0.5 mg/kg a day) (Table 1). ACTH, beta-endorphin and CRH levels were not affected by the administration of dexamethasone (0.05 or 0.1 mg/kg) per os. Since the patient had undergone an operation with Whipple's procedure, the absorption of dexamethasone might be limited. However, we observed an increase in the dexamethasone concentration in plasma after administration of the agent. In order to examine whether much higher doses of the dexamethasone affected the level of these hormones, 0.2 mg/kg of dexamethasone was administered per os. ACTH and beta-endorphin were decreased by the admin.

### Table 1. Endocrinological findings

<table>
<thead>
<tr>
<th></th>
<th>0600</th>
<th>0900</th>
<th>1500</th>
<th>2300</th>
<th>Normal range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRH (pg/ml)</td>
<td>48.4</td>
<td>44.3</td>
<td>41.2</td>
<td>43.0</td>
<td>(5.7±0.6)</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>190</td>
<td>186</td>
<td>191</td>
<td>222</td>
<td>(17.4±7.3)</td>
</tr>
<tr>
<td>beta-endorphin (pg/ml)</td>
<td>1500</td>
<td>1400</td>
<td>1300</td>
<td>990</td>
<td>(17.0±5.0)</td>
</tr>
</tbody>
</table>

2) Secretin Test (3U/kg/hr for 15 min iv from 0 to 15 min) (Normal range*)

<table>
<thead>
<tr>
<th>min.</th>
<th>0</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin (pg/ml)</td>
<td>53</td>
<td>40</td>
<td>48</td>
<td>44</td>
<td>44</td>
<td>45</td>
<td>41</td>
</tr>
</tbody>
</table>

3) Oral glucose (75-g) Tolerlance Test (Normal range*)

<table>
<thead>
<tr>
<th>min</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (mg/100 ml)</td>
<td>76</td>
<td>151</td>
<td>140</td>
<td>111</td>
<td>101</td>
</tr>
</tbody>
</table>
| IRI (µU/ml) | 68 | 178 | 189 | 47 | 49 | (15±5)

* indicates mean±S.D. of hormone levels in plasma obtained at 0600 a.m. (CRH, ACTH and beta-endorphin) or at 0800 a.m. (gastrin, glucose I. R. I. (immunoreactive insulin)) in 34 normal subjects.
tration, but the plasma CRH concentration was not affected by the administration. In this condition, SMS-201-995 was also administered subcutaneously and further decreased plasma ACTH and beta-endorphin. In addition, plasma CRH was also decreased by the administration (Fig. 1).

Examinations with computed tomography (CT) revealed the presence of multiple metastatic tumors in liver (Fig. 2) but did not disclose any enlargement of the pituitary gland. Cytology of the specimens obtained from metastatic tumors in liver revealed the presence of malignant cells derived from a pancreatic islets. In order to evaluate the function of the tumor cells, immunoreactivity to anti-ACTH, anti-beta-MSH, anti-beta-endorphin and anti-CRH antibodies was examined. ACTH, beta-MSH and beta-endorphin were recognized in the metastatic tumor cells (Fig. 3). However, anti-CRH antibody did not recognize the antigen in the cells. Immunocytochemical studies for ACTH, beta-endorphin and CRH were also performed on the initial pancreatic tumor, and we obtained results similar to those obtained in metastatic tumour cells. No antibody recognized any peptide in the cells derived from normal human pancreatic islets.

**Effects of Acute Administration of SMS 201–995**

Acute administration of SMS 201–995...
Fig. 2. Computed tomographic scanning of liver.
The right panel shows scanning at the time of admission and the left one shows an image in the same patient 3 years before admission.

Fig. 3. (A)
Fig. 3. Immunocytochemical localization of ACTH, beta endorphin and beta-MSH. The panels (×400) (from A to C) show immunostaining for ACTH, for beta-endorphin and for beta-MSH of the tumor.
(200 µg, i.m.) decreased not only serum GH but also plasma ACTH. However, these hormones increased 24-hr after the administration of SMS 201–995. Serum prolactin was not significantly decreased. Subcutaneous administration of SMS 201–995 (150 µg) decreased plasma ACTH. Plasma CRH was also decreased by the injection. In this case, CRH again increased 6-hrs after the injection (Fig. 4).

**Effects of Chronic Administration of SMS 201–995**

SMS 201–995 was administered to the patient subcutaneously for 70 days. The dose of the agent was 450–500 µg/day and injections were given three times a day (7:00 a.m., 14:00 p.m., and 21:00 p.m.). The administration continuously decreased plasma ACTH, beta-endorphin and alpha-MSH. In addition to the decrease in these

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**Fig. 4. Effects of 201–995 on plasma ACTH, GH, PRL and CRH levels.**

The left panel shows the acute effect of i.m. injection of SMS 201–995 (200-µg), and the right panel shows the effect of s.c. injection of SMS 201–995 (150-µg). The arrows indicate the time of administration of each agent.

**Fig. 5. Effects of chronic administration of SMS 201–995 on plasma levels of CRH, ACTH, beta-endorphin and alpha-MSH.**

SMS 201–995 (450-µg or 500-µg/day) was administered s.c. Injection were given 3 times (07:00 a.m., 14:00 p.m. and 21:00 p.m.) a day. Blood samples were drawn at 07:00 a.m. before daily injection of SMS 201–995. SRIF denotes SMS 201–995.
hormone levels in plasma, a decrease in plasma CRH was observed (Fig. 5). During the treatment with SMS 201–995 for 70 days, pigmentation of the lips and tongue disappeared.

Discussion

It is well documented that plasma ACTH levels are high in patients with Nelson’s syndrome (Bondy, 1986). We could not define whether the patient presented in this report had Nelson’s syndrome or not. Since the patient had undergone bilateral adrenalectomy, overproduction of ACTH and beta-endorphin in the pituitary might occur even though the pituitary tumor was not revealed in CT scanning. However, evidence of the presence of immunoreactivities against ACTH and its related peptides in tumor cells strongly suggested that the high level of plasma ACTH was caused by ectopic (metastatic) tumors in the liver. The plasma CRH concentration is also known to be increased in certain patients with Nelson’s syndrome (Suda et al., 1985). Since we do not know the CRH level before adrenalectomy in this patient, it was impossible to define whether the high level of CRH was induced by adrenalectomy or was due to hypersecretion which was associated with MEN-I (Lips et al., 1984, Yamaguchi et al., 1980). In either case, the high levels of plasma ACTH and CRH after adrenalectomy suggested that the patient was in a state similar to that in Nelson’s syndrome. The molar ratio of ACTH to beta-endorphin in this patient was 0.16 (mean of four different simultaneous measurements), which was significantly lower than that observed in normal subjects (Table 1). The reason why the molar ratio decreased in this patient was not clear.

It has been reported that the increased secretion of ACTH from the pituitary is able to be inhibited by the administration of SRIF or its long-acting analogue in Nelson’s syndrome (Tyrrell et al., 1975, Benker et al., 1976, Lamberts et al., 1989, Reichlin, 1983). However, it was uncertain whether SRIF inhibits secretion of CRH or not. We observed that a decrease in plasma ACTH was associated with a decrease in plasma CRH in both studies with acute and chronic administration of SMS 201–995. These results suggested that SMS 201–995 inhibited secretion not only of ACTH but also of CRH. In addition to these observations, dexamethasone-unsuppressible secretion of CRH was inhibited by the agent, which accompanied a further decrease in plasma ACTH. The results suggested that the SMS 201–995-induced decrease in plasma ACTH might be partly dependent on the agent-induced inhibition of the overproduction of CRH.

Immunocytochemical studies showed the presence of ACTH, beta-endorphin and beta-MSH in the metastatic tumor cells, suggesting that the precursor molecule of ACTH, which is known as proopiomelanocortin (POMC), had been synthesized in the tumor cells (Krieger, 1983). CRH is known to stimulate the synthesis of POMC in pituitary cells (Vale et al., 1981). We observed a parallel decrease in plasma CRH and these peptides during the administration of SMS 201–995. The results indicated that the SMS 201–995-induced decrease in plasma ACTH was not only caused by direct inhibition of ACTH release but also resulted from the inhibition of CRH-induced production of POMC in the tumor cells. However, further studies, such as in vitro experiments on peptide secretion from tumor cells, and measurement of the bioactivity of plasma CRH will be required to clarify the mechanism of SMS 201–995 inhibition of the secretion of these peptides.

As mentioned above, we postulated that overproduction of CRH plays an important role in the stimulation of hypersecretion of
ACTH from tumor cells in this case. The site of CRH secretion, however, could not be identified. We had supposed that the metastatic tumors were sites of CRH production. However, we failed to observe a positive reaction with anti-CRH antibody in immunocytochemical studies on the metastatic tumor cells. The result indicated that the excess CRH produced was held in cells other than the tumor cells, as is observed in patients with Nelson’s syndrome (Bondy, 1986).

In this study, we reported that the long-acting SRIF analogue (SMS 201–995) is effective in inhibiting CRH and ACTH secretions in a patient with ectopic ACTH-producing tumor associated with MEN-I. Although long-term administration will be required to evaluate the clinical usefulness of the agent (Bertagna et al., 1989, Lamberts et al., 1987, Barken et al., 1988), we observed the disappearance of pigmentation following administration of the agent for 70 days, suggesting that this agent may be useful in treating hypersecretion of ACTH and CRH.

References


