Effects of a Long-Acting Somatostatin Analogue on Pituitary-Adrenocortical Secretion in Normal Human Subjects

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Abstract

A potent and long-acting somatostatin analogue, SMS 201-995 (SMS) is currently employed for the treatment of various diseases with hypersecretion of hormones such as acromegaly and gastrinoma. The suppressive effects of SMS are also reported on the other pituitary and gastrointestinal hormones. The corticotropic-adrenocortical axis is a crucial hormonal complex in maintaining normal activity and life itself. In this study, the effects of SMS on corticotropic-adrenocortical functions were studied, since the effects of SMS on this hormonal axis are not well established. Seven normal males received a sc injection of 100 μ g SMS or placebo at 0830 h and 100 μ g synthetic human corticotropin-releasing hormone (hCRH) intravenously (SMS-hCRH study). Five of the 7 subjects were given an injection of a synthetic (1–24) ACTH (250 μ g or 63 μ g) at 0900 h after 100 μ g SMS or a placebo at 0830 h (SMS-ACTH study). Blood samples were drawn at –30, 0, 15, 30, 60, 90 and 120 min after the hCRH injection for the determination of ACTH and cortisol in the SMS-hCRH study, and cortisol and aldosterone in the SMS-ACTH study. Although significant rises in plasma ACTH and cortisol levels were observed regardless of the preinjection of SMS, their responses to hCRH were significantly lower with the pretreatment with SMS than without SMS. A significant increase in plasma cortisol and aldosterone was observed in response to synthetic ACTH with both ACTH alone and the combined administration of SMS and ACTH, at either dose of ACTH. However, no significant difference in cortisol and aldosterone secretion was detected with and without SMS. These results suggest that a single sc administration of SMS suppresses CRH-induced ACTH and cortisol secretion, but not ACTH-induced cortisol and aldosterone, and also favor the possibility that somatostatin is involved in the regulation of ACTH secretion in man. Based on the present observations, it may be worthwhile to pay attention to adrenocortical function in patients under long-term therapy with SMS. However, further study is required to establish the exact effects of SMS on adrenocortical secretion with a smaller amount of ACTH than used in this study.

Received November 22, 1989

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This work was supported in part by a Research Grant for Intractable Diseases from the Japanese Ministry of Health and Welfare, a research grant from the Japanese Ministry of Education, Science and Culture and a research grant from the Sankyo Foundation of Life Science.

Somatostatin, initially isolated from the hypothalamus (Vale *et al.*, 1975), as a potent growth hormone (GH) or somatotropin release inhibiting factor (SRIF), is known to be produced in a number of other tissues such as pancreas (Newgard and Holst 1981; Ertan *et al.*, 1981; Ito *et al.*, 1982a; Ito *et al.*, 1982b), gut (Itoh *et al.*, 1982a; Fitz-Patrick and Patel, 1981; McIntosh, 1985) and placenta (Lee *et al.*, 1982). SRIF suppresses not only GH but also many other hormones including gonadotropin (Millar *et al.*, 1982), prolactin (Skamene and Patel, 1984), gastrin (Lucey *et al.*, 1984), glucagon (Souquet *et al.*, 1983; Tanaka *et al.*, 1984a) and thyroid hormone (Ahren *et al.*, 1978). Based on these observations, therapeutic approaches to the treatment of various diseases accompanied by hypersecretion of hormones with SRIF have been made but unsuccessfully, because of the very short half-disappearance time of SRIF in plasma (Skamene and Patel 1984) and of the rebound secretion after the transient suppression of the hormone (Gill *et al.*, 1982). For this reason, a number of the synthetic analogues of SRIF such as SMS 201-995 (SMS) and WY-41,747 (Dimitriadis *et al.*, 1983) have been developed. Among these, long term subcutaneous injection of SMS has been used in patients with such diseases as acromegaly (Pieters *et al.*, 1986) and gastrinoma (Bonfils *et al.*, 1986) with effective therapeutic results because of its much longer plasma disappearance half time (Pozo *et al.*, 1986). Adrenocorticotropin (ACTH)-adrenocortical hormone and thyrotropin (TSH)-thyroid hormones are critically important in maintaining daily activity and eventually life itself. Recently we have reported the suppressive effect of SMS on the secretion of TSH in man (Itoh *et al.*, 1988; Masuda *et al.*, 1989). However, the effect of SMS on the corticotropic-adrenocortical axis has not been established yet in man. The present study was undertaken to study the effect of SMS on ACTH, cortisol and aldosterone in man. It is well established that there is a diurnal rhythm in ACTH-

adrenocortical hormones (Orth *et al.*, 1967; Tanaka *et al.*, 1978; Watabe *et al.*, 1987a) whose concentration is highest in the early morning and declines gradually thereafter. Because it is difficult to distinguish this normal decline and suppression by SMS, corticotropin-releasing factor (CRH) (Vale *et al.*, 1981; Vale *et al.*, 1983; Shibahara *et al.*, 1983) and (1–24) ACTH (Wood *et al.*, 1965), which specifically stimulate corticotroph and the adrenal cortex, respectively, were used.

Materials and Methods

Subjects and protocol

Seven normal males participated in the study after obtaining their informed consent. The experiments were performed according to the following 6 protocols, after an overnight fast. SMS or a placebo was given sc at 0830 h and synthetic human corticotropin-releasing hormone (hCRH) or synthetic (1–24)ACTH iv at 0900 h, respectively.

1. SMS-hCRH study

All seven subjects (weight 65.6 ± 3.0 kg, age 34.0 ± 1.9 yrs old) received (1) 100 μ g SMS at –30 min and 100 μ g hCRH at 0 min, (2) placebo at –30 min and 100 μ g CRH at 0 min.

2. SMS-ACTH study

Five of the seven subjects (weight 63.4 ± 3.1 kg, age 34.2 ± 2.7 yrs old) received (1) 100 μ g SMS at –30 min and 250 μ g synthetic (1–24) ACTH at 0 min, (2) placebo at –30 min and 250 μ g ACTH at 0 min, (3) 100 μ g SMS at –30 min and 63 μ g ACTH at 0 min, (4) placebo at –30 min and 63 μ g ACTH at 0 min.

The experiments were separated by more than a week. Blood specimens were obtained at –30, 0, 15, 30, 60, 90 and 120 min for the determination of ACTH and cortisol in the SMS-hCRH study and cortisol and aldosterone in the SMS-ACTH study.

Materials

hCRH was obtained from Peptide Institute (Osaka, Japan) and prepared as reported previously (Tanaka *et al.*, 1983). SMS was kindly provided by Sandoz Co., Ltd., and synthetic (1–24) ACTH was purchased from Daiichi

Pharmaceutical Co., Ltd., Tokyo, Japan. As a placebo for SMS or ACTH, the same volume of normal saline was used in the control studies.

Determinations of hormones

Plasma ACTH was assayed after the extrac-

tion of the sample with a Sep-Pak C18 cartridge (Waters Associates, Milford, MA, USA) in a specific radioimmunoassay (RIA) as described previously (Watanabe *et al.*, 1987b). The mean recovery of ACTH was $77.2 \pm 6.2\%$ at an ACTH concentration of 30.0 pg/ml plasma and the results were corrected for recovery. The intra- and inter-assay coefficient of variation (CV's) were 8.4 and 10.8%, respectively. Plasma cortisol was assayed with a RIA kit obtained from Eiken Immunochemical Lab., Tokyo, Japan as reported (Tanaka *et al.*, 1984b) and the intra- and inter-assay CV's were 4.3 and 6.2%, respectively. Aldosterone was determined with a RIA kit obtained from Sorin Biomedica, France and the intra- and inter-assay CV's were 8.4 and 12.1%, respectively. All samples from an individual man were analyzed at the same time in duplicate in each assay.

Statistical analysis

When multiple comparisons were made, two-way analysis of variance was performed followed by Student's paired *t*-test. Student's paired *t*-test was used to compare the values between the same time points during the experiments with and without SMS. The area under the concentration curve (AUC) was determined by calculating the area under the hormone response curve above the basal hormone level. The results were described as the mean $\pm$ SEM unless otherwise indicated.

Results

Plasma ACTH responses to hCRH without SMS are shown in Fig. 1 (upper panel). The mean plasma

Fig. 1. Mean ($\pm$ SEM) plasma ACTH (upper panel) and cortisol (lower panel) levels before and after iv injection of hCRH without pretreatment (open circles) and those with pretreatment with SMS 201-995 (closed circles) in seven normal subjects. The asterisks above and below the points indicate the statistical significance of the values between the points and the value at -30 min and those within the curves, between the points at the corresponding times. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

ACTH level rose significantly ($F=38.0$, $p<0.01$) from the basal level (28.2 ± 5.8 and 28.0 ± 4.9 pg/ml at -30 and 0 min, respectively) to a peak level of 78.6 ± 9.5 pg/ml at 30 min after the injection of hCRH. With the preinjection of SMS, the mean basal plasma ACTH level was 28.5 ± 5.8 and 23.4 ± 5.4 pg/ml at -30 and 0 min, respectively and it rose ($F=29.8$, $p<0.01$) to a peak of 53.5 ± 8.2 pg/ml at 30 min after hCRH injection. The mean ACTH levels after the hCRH injection were significantly lower at time 30 and 60 min with preinjection of SMS than without SMS (Fig. 1. upper panel).

The mean basal cortisol levels (11.9 ± 0.9 and 11.1 ± 1.1 $\mu\text{g/dl}$ at -30 and 0 min, respectively) rose significantly ($F=54.6$, $p<0.01$) to a peak level of 23.3 ± 2.8 $\mu\text{g/dl}$ at 60 min after hCRH alone injection, and also significantly rose ($F=74.5$, $p<0.01$) from the mean basal level (11.1 ± 1.1 and 9.8 ± 0.9 $\mu\text{g/dl}$, at -30 and 0 min, respectively) to a peak level of 16.6 ± 1.6 $\mu\text{g/dl}$ at 60 min after hCRH injection when pretreated with SMS (Fig. 1. lower panel). The mean cortisol levels appeared to be lower from 30 to 120 min during the SMS-treatment than placebo-treatment experiment, although statistical significance was found only at time 90 min (Fig. 1. lower panel).

The integrated responses estimated as the mean areas under the concentration curve (AUC) for ACTH were 3784 ± 416 and 2223 ± 312 pg/ml·min in the experiments without and with SMS, respectively (Fig. 2). AUC's for cortisol were 948 ± 154 and 482 ± 123 $\mu\text{g/dl}\cdot\text{min}$, in the experiments without and with SMS, respectively (Fig. 2). Significant differences ($p<0.05$) were demonstrated in the mean AUC for both plasma ACTH and cortisol.

Effects of iv injection of 250 μg or 63 μg synthetic ACTH on plasma cortisol concentrations are shown in Fig. 3. After the injection of 250 μg or 63 μg ACTH alone, the mean plasma cortisol levels rose significantly ($F=114.6$, $p<0.01$ for 250 μg ACTH and $F=120.5$, $p<0.01$ for 63 μg ACTH) (Fig. 3A

Fig. 2. Mean ($\pm$ SEM) AUC for plasma ACTH (left panel) and cortisol (right panel) after the injection of hCRH alone (open columns) or SMS 201-995 plus hCRH (shaded columns) in seven normal subjects. The asterisks indicate significant differences between values for hCRH and the combined injection of hCRH and SMS 201-995.

*, $p<0.05$.

and 3B). The responses of plasma cortisol to ACTH (250 or 63 μg) with preinjection of SMS were also statistically significant ($F=160.6$, $p<0.01$ and $F=36.2$, $p<0.01$, respectively, Fig. 3A and 3B). However, there was no significant difference between the plasma cortisol levels in the SMS-treated and SMS-untreated experiments, except at 15 min at ACTH doses of 250 μg ($p<0.05$) (Fig. 3A).

Effects of iv injection of 250 μg and 63 μg ACTH on plasma aldosterone concentrations are shown in Fig. 3C and 3D, respectively. The mean plasma aldosterone levels rose significantly after the iv injection of ACTH alone ($F=16.6$, $p<0.01$ for 250 μg ACTH and $F=22.4$, $p<0.01$ for 63 μg ACTH) (Fig. 3C and 3D). The response of plasma aldosterone to ACTH (250 or 63 μg)

Fig. 3. Mean ($\pm$ SEM) plasma cortisol (A and B) and aldosterone (C and D) levels before and after iv injection of ACTH without pretreatment (open circles) and those with pretreatment with SMS 201-995 (closed circles) in five normal subjects. Statistical analysis of the data shown in this figure is described in the text.

with preinjection of SMS was also statistically significant ($F=13.5$, $p<0.01$ and $F=14.3$, $p<0.01$, respectively, Fig. 3C and 3D). There was, however, no significant difference between in plasma aldosterone levels in the SMS-treated and SMS-untreated experiments.

AUC for cortisol were 1824 ± 235 and 1941 ± 246 $\mu\text{g/dl} \cdot \text{min}$ after the injection of 250 and 63 μg ACTH alone, respectively, and 2027 ± 150 and 1890 ± 312 $\mu\text{g/dl} \cdot \text{min}$ after the administration of 250 and 63 μg

ACTH with preinjection of SMS, respectively (Fig. 4). AUC for aldosterone were 1850 ± 436 and 1390 ± 271 $\text{pg/ml} \cdot \text{min}$ after 250 and 63 μg ACTH alone, respectively, and 1475 ± 387 and 1493 ± 417 $\text{pg/ml} \cdot \text{min}$ after 250 and 63 μg ACTH with pretreatment by SMS, respectively (Fig. 4). No significant differences were demonstrated in the mean AUC for plasma cortisol and aldosterone.

Fig. 4. Mean ($\pm$ SEM) AUC for plasma cortisol (upper panel) aldosterone (lower panel) after the injection of ACTH alone (open columns) or SMS 201-995 plus ACTH (shaded columns) in five normal subjects.

Discussion

Among a number of hormonal complexes, the ACTH-adrenal cortex and TSH-thyroid axes are the most important ones in terms

of maintaining normal daily activity and life itself in man and animals. SRIF and the synthetic agonists including SMS, are known to suppress the secretion of a number of hormones in normal subjects and in patients with hormonally functioning tumors as outlined in the Introduction. We have recently reported in normal subjects that 30 to 45 min after a single sc administration of 100 μ g SMS, the plasma concentration of SMS reaches its maximal and that significant suppression of TSH secretion lasts for at least 8 hours (Itoh *et al.*, 1988). We have also shown that GH secretion is suppressed at least 4 hours in normal male subjects after the same doses of SMS (Hashida *et al.*, 1986). Based on these observations, SMS was administered in this study 30 min prior to the injection of CRH or ACTH. The present results indicate that a single sc injection of SMS (100 μ g) clearly suppresses CRH-induced ACTH and cortisol secretion when evaluated by their plasma levels and AUCs. The present data are in good agreement with the *in vitro* study in which both basal (Reisine, 1985) and CRH-stimulated (Richardson, 1983; Suda *et al.*, 1984; Kraicer *et al.*, 1985) ACTH secretion have been shown to be suppressed by SRIF.

SRIF has been shown to suppresses aldosterone (Aguilera *et al.*, 1981; Jones *et al.*, 1984; Rebuffat *et al.*, 1989) secretion from the rat adrenal cortex. In the present study, however, no significant suppression by SMS was observed in 63 μ g or 250 μ g ACTH-induced aldosterone secretion. The most likely explanation for this is that the amounts of synthetic ACTH used in our study are greater than those necessary to provide clear answers to our questions, since there was no significant difference in the response of aldosterone at the two ACTH doses. As to the direct action of SRIF or its agonistic derivatives, there is little information in the literature, although ACTH-induced cortisol secretion is unaffected by the infusion of SRIF in man (Johnston *et al.*, 1982). In another study on the rat, SRIF had no effect on ACTH-induced corticoste-

rone secretion (Robba *et al.*, 1986). Thus, the suppression of CRH-induced cortisol secretion by SMS observed in the present study probably results from the attenuated secretion of ACTH by the pretreatment with SMS and not from the direct inhibitory action on the adrenal cortex. However, experiments with the smaller doses of ACTH are required to establish a direct suppressive action by SMS on adrenocortical secretion in man.

For therapeutic purposes, the amounts of SMS that have been used are much greater (Jackson *et al.*, 1986; Vinik *et al.*, 1986) than those tested in the present study and also SMS was injected every 8 to 12 hours for more than a month in many of the studies (Lamberts *et al.*, 1985; Lamberts and Pozo, 1986). Thus, the exact effects of SMS on adrenocortical function in the current therapeutic regimens remains to be established. It might be well to pay attention to thyroid and adrenocortical function in patients particularly under the long-term therapy with potent and long-acting agonist of SRIF such as SMS. The present study also suggests the possibility that SRIF could be one of the factors in regulating ACTH secretion since SRIF is synthesized in the hypothalamus and secreted at high concentration into the hypophyseal portal vein (Patel *et al.*, 1980) and also suggests possible therapeutic application of SMS to patient with hypersecretion of ACTH, such as Cushing's disease, Nelson's syndrome or ectopic ACTH producing tumors.

Acknowledgements

We are grateful to Miss Keiko Noguchi, Mrs. Yumiko Hiromura and Miss Tamiko Komatsu for their excellent technical assistance. We also thank the NIDDK and the National Hormone and Pituitary Program for generously supplying synthetic (1-39) hACTH used for RIA. The authors wish to thank Sandoz, Ltd., Basle, Switzerland for generously supplying SMS 201-995.

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