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 μg SMS or placebo at 0830 h and 100 μg 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 μg or 63 μg) at 0900 h after 100 μg 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.

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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). Adrenocorticotropic hormone (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 corticotropin-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 μg SMS at -30 min and 100 μg hCRH at 0 min, (2) placebo at -30 min and 100 μg hCRH 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 μg SMS at -30 min and 250 μg synthetic (1–24) ACTH at 0 min, (2) placebo at -30 min and 250 μg ACTH at 0 min, (3) 100 μg SMS at -30 min and 63 μg ACTH at 0 min, (4) placebo at -30 min and 63 μg 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 extraction 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±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 Immunological 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±SEM unless otherwise indicated.

**Results**

Plasma ACTH responses to hCRH without SMS are shown in Fig. 1 (upper panel). The mean plasma
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 μg/dl at -30 and 0 min, respectively) rose significantly (F=54.6, p<0.01) to a peak level of 23.3±2.8 μg/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 μg/dl, at -30 and 0 min, respectively) to a peak level of 16.6±1.6 μg/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 μg/dl·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 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)....
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 µg/dl.min after the injection of 250 and 63 µg ACTH alone, respectively, and 2027±150 and 1890±312 µg/dl.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 pg/ml.min after 250 and 63 µg ACTH alone, respectively, and 1475±387 and 1493±417 pg/ml.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.
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 μg 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 μg) 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 suppress 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 μg or 250 μg 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-
ronone 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 regimes 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 hypophysal 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.

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