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Inhibition of pentagastrin-stimulated acid secretion after subcutaneous administration of a new somatostatin analogue

I WHITEHOUSE, C BEGLINGER, G RÜTTIMANN, AND K GYR

From the Division of Gastroenterology and Department of Research, University Hospital, Basel, Switzerland

SUMMARY Somatostatin, a peptide present in hypothalamus, gastric mucosa, and pancreas suppresses several gastrointestinal functions. Its short half life has prevented clinical use. We have therefore evaluated the effect of subcutaneous administration of a new synthetic somatostatin analogue, in comparison with a placebo, on pentagastrin stimulated acid secretion in six healthy volunteers. On different days, acid secretion was measured continuously, after a basal 30 minutes, for six hours during 3 μg/kg/h of intravenous pentagastrin. Acid secretion was measured with a marker technique (0·1% phenol red) to correct for duodenal volume loss. Blood was drawn in regular intervals to measure plasma somatostatin concentrations by radio immunoassay. One hour after starting the pentagastrin infusion, a single subcutaneous injection of either 100 μg somatostatin analogue, or placebo (isotonic saline) was given. In a follow up study, somatostatin was given subcutaneously in a dose of 200 μg. No difference in efficacy was observed between the two doses. A single subcutaneous injection of the somatostatin analogue significantly suppressed acid secretion for five hours ($p<0·01$). Maximal inhibition was approximately 75%. Mean elimination half life of the analogue was approximately 80 minutes. We suggest that the new somatostatin analogue might be useful for clinical use.

Because of its potent inhibition of different gastrointestinal functions, the use of somatostatin (SMS), a tetradecapeptide, originally isolated from bovine and porcine hypothalammi[1 2] has been advocated in the treatment of some gastrointestinal disorders such as peptic ulcer haemorrhage[3 4] and gastrointestinal endocrine tumours.[5–7] Its very short half life of two to three minutes prevents its administration other than by intravenous infusion[3 8] and thus the long term clinical use. The search for appropriate analogues is therefore ongoing.

Somatostatin 201-995 (SMS) is a synthetic octapeptide analogue of natural SRIF and possesses many of the pharmacological properties of the natural peptide. Plasma half life was found to be approximately 45 minutes after intravenous application.[9] We have recently shown that intravenous administration of SMS dose dependently inhibited pentagastrin stimulated acid secretion in man.[10] It appeared to have a longer duration of action than SRIF.

Address for correspondence: Professor Klaus Gyr, Division of Gastroenterology, University Hospital, CH-4031 Basel, Switzerland.

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The purpose of this study was to test the efficacy of SMS against placebo in inhibiting pentagastrin stimulated gastric acid secretion after single subcutaneous injections.

Methods

SUBJECTS

One healthy woman and five healthy men, median age 22·5 years (range 21–24 years), median weight 74 kg (range 53–93 kg), with no history of gastrointestinal disease were investigated. The protocol was approved by the local Ethical Committee and the study done according to the guidelines of the declaration of Helsinki/Tokyo. All participants gave informed written consent.

EXPERIMENTAL DESIGN

Afer an overnight fast a double lumen gastric tube was positioned in the antral part of the stomach under fluoroscopic control. Phenol red (0·1%), a non-absorbable marker, was used to correct for duodenal volume loss[11] and instilled into the stomach close to the cardia at a flow rate of 200 ml/h.

Gastric acid was aspirated in 15 minute periods by continuous mechanical suction. Every five minutes 10 ml air was injected through the tube to maintain patency. After a basal period of 30 minutes, pentagastrin dissolved in 0·154 mol saline was administered as a continuous intravenous infusion in a dose of 3 μg/kg/h and set at a flow rate of 25 ml/h for six hours. This dose of pentagastrin has been shown to produce near maximal acid secretion.[12] After 60 minutes of pentagastrin infusion, a single subcutaneous injection of either 100 μg SMS or placebo (isotonic saline) was given in randomised order into the deltoid region of the right arm. This dose of SMS was chosen from pilot experiments in which nearly identical plasma concentrations were achieved after 35 μg/h intravenously and 100 μg subcutaneously administered SMS. The intravenous dose has been shown to significantly suppress pentagastrin stimulated gastric acid secretion.[10] Blood samples for analysis of plasma SMS concentrations were taken from an indwelling catheter in a cubital vein at baseline and in regular intervals thereafter. The plasma was immediately centrifuged at 3000 rpm at 4°C and the supernatant stored at −20°C until assayed.

In a follow up study the same volunteers received 200 μg of SMS subcutaneously. The same experimental protocol was used as described before.

DETERMINATIONS

The volume of all aspirates was measured and the gastric juice analysed for the concentration of H^+ and phenol red. Acid concentration was estimated by titration with 0·01 mol sodium hydroxide to an end point of pH 7·0 using an electrometric autotitrator (Methrom, Herisau, Switzerland). Phenol red concentration was determined by photometry at 546 nm wave length.

Somatostatin concentration in non-extracted plasma was determined by a sensitive and specific radioimmunoassay. Briefly, the antiserum originated from a mouse which had been immunised with the peptide conjugated to haemocyanin. As a tracer, the (Tyr^1)-analogue of SMS was iodinated and purified on HPLC. Detection was limited to 0·02 ng/ml plasma. All samples were assayed in duplicate.[13]

Pentagastrin (Peptavlon^R) was obtained from ICI Pharma Luzern, Switzerland. Somatostatin was provided by Sandoz Ltd, Basel, Switzerland.

STATISTICS

For each individual, the mean values of volume secretion and acid output during the last two collection periods under pentagastrin alone were calculated. The total acid output or total gastric fluid secretion per five hours during SMS or placebo administration was then calculated. The results, expressed as mean±SEM, were evaluated by Student's *t* test for paired data. The significance level was set at $p<0·05$. For the evaluation of the plasma SMS concentrations, a one compartment model was used with drug elimination in first order fashion. Disappearance half life was calculated by least square regression analysis.

Results

During infusion of pentagastrin, a significant increase in gastric acid secretion was found. Comparison of acid secretion in the three experiments under pentagastrin alone (60–90 min) show no significant difference. The mean values are given in the Table. In our study no significant decrease of acid concentration and of acid secretion was observed over the six hours of the placebo administration, although both parameters had a tendency to decline towards the end of the experiment (Fig. 1).

A single subcutaneous injection of SMS (100 μg) decreased pentagastrin stimulated acid secretion within 15 minutes after administration. The time course and extent of inhibition of gastric acid output are given in Figure 1. Somatostatin inhibited gastric acid secretion throughout the five hour observation

Table Gastric fluid and acid output after subcutaneous somatostatin or placebo in six healthy volunteers

	Fluid secretion			*Acid output*		
	Placebo	*Somatostatin*		*Placebo*	*Somatostatin*	
		100 ug sc	*200 ug sc*		*100 ug sc*	*200ug sc*
Control period (pentagastrin alone)	135±10 ml/15 min	130±11 ml/15 min	126±11 ml/15 min	10±2 mmol/15 min	9±2 mmol/15 min	9±2 mmol/15 min
Total output/5 hours after subcutaneous somatostatin or placebo	2525±195 ml/5 h	1856±138* ml/5 h	1746±99* ml/5 h	175±22 mmol/5h	87±18† mmol/5 h	84±20† mmol/5 h

Data are mean±SEM, N=6. * Indicates $p<0·05$. † Indicates $p<0·001$ (significant reduction of gastric fluid and acid output respectively, compared with placebo).

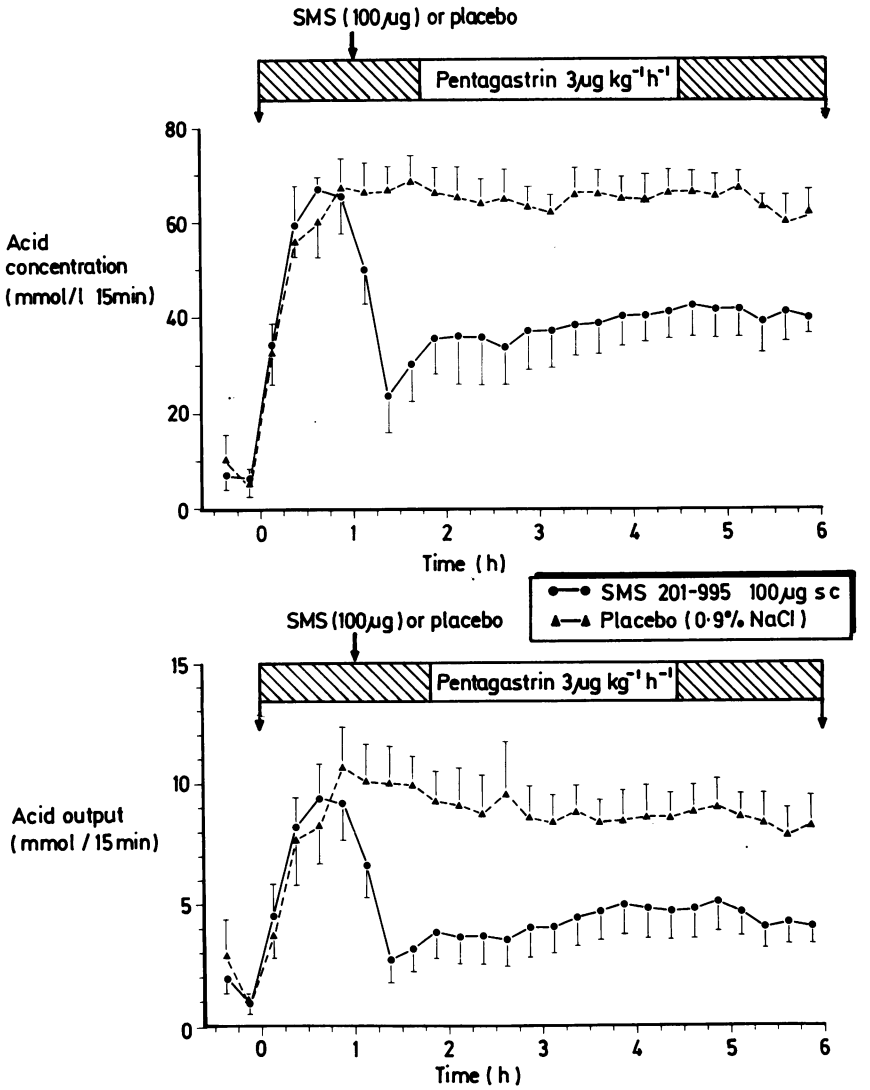

Fig. 1 *Time curve of gastric acid concentration (mmol/l/15 minutes) and gastric acid output (mmol/15 minutes) during a six hour continuous infusion of pentagastrin (3 µg/kg/h) after subcutaneous injection of either somatostatin or placebo (data are mean±SEM, N=6).*

period. No significant decrease in the degree of inhibition was observed at the end of the experiment (total acid output in the first and the last hour after SMS was 16±4 and 16±3 mmol/h respectively) (Fig. 2). The percentage reduction of acid output for the 15 minute period of maximal inhibition after SMS was 75±4% compared with the corresponding period after placebo injection. Total acid output per five hours was significantly suppressed by SMS ($p<0·001$, Table).

Comparison of gastric fluid secretion during the control period shows no significant difference in the three experiments (Table). Tota fluid secretion per five hours was significantly suppressed by somatostatin compared with placebo administration ($p<0·05$, Table). The maximum percent reduction (15 minute period of maximal inhibition) in gastric volume secretion amounted to 35±3% for 100 µg of sc SMS compared with the respective control period ($p<0·05$).

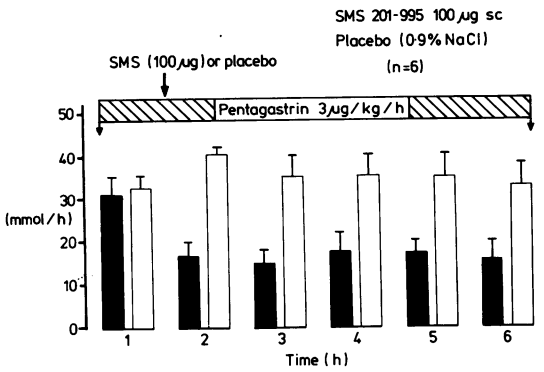

Fig. 2 *Total hourly acid output in mmol/h (mean±SEM) in the same healthy volunteers. Acid secretion is significantly suppressed for each hour after somatostatin administration ($p<0·05$ to $p<0·01$).*

Two hundred micrograms somatostatin produced the same inhibition in acid output and fluid secretion as observed with the lower dose (Table).

The time course of plasma somatostatin is given in Figure 3. Immunoreactive somatostatin increased rapidly after subcutaneous injection of 100 µg reaching a mean peak concentration of 3·5±0·5 ng/ml after 14·0±4·5 min. After five hours, plasma somatostatin concentrations had decreased to 0·4±0·1 ng/ml. The respective results for the 200 µg dose were: peak concentration=11·5±1·4 ng/ml after 20·8±4·6 min; plasma somatostatin concentrations after five hours: 1·1±0·1 ng/ml. The mean elimination half lifes ($t_{\frac{1}{2}}$) of somatostatin after subcutaneous administration of 100 or 200 µg determined from each individual were 1·33±0·21 and 1·37±0·17 hours respectively. The lack of correlation between acid concentration and plasma somatostatin concentrations is illustrated by the following data: y=52·4–2·64 x, r=0·16, where y represents acid concentration and x represents plasma somatostatin concentrations at various time points.

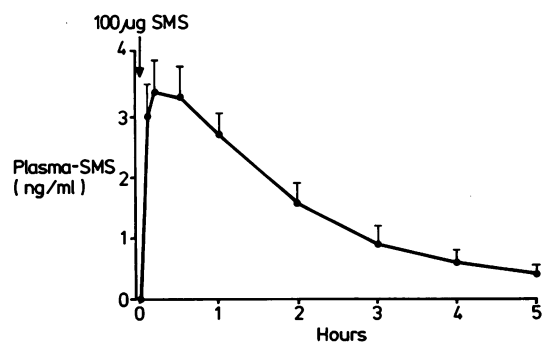

Fig. 3 *Plasma somatostatin concentrations (ng/ml) in resonse to 100 µg subcutaneous somatostatin. Data are mean±SEM.*

Discussion

The results of the present study can be summarised as follows: (1) Subcutaneous administration of the synthetic SMS analogue SMS 201-995 produced a marked inhibition of pentagastrin-stimulated acid secretion. (2) The percentage reduction of acid output was in the same order of magnitude as observed with intravenous SMS or with natural SMS-14.[1 9] (3) A single injection significantly suppressed gastric acid secretion for the five hour duration of the experiment.

Natural SMS is a tetradecapeptide with a variety of actions. It has been tested successfully as a therapeutic agent in peptic ulcer haemorrhage,[2–4] pancreatic and intestinal fistula,[14] gastrointestinal endocrine tumours.[5–7] Favourable responses have been observed in patients with diabetes mellitus[15] and carcinoid flushing.[16 17] The clinical value of SMS has, however, been limited owing to its very short duration of action and by its lack of specificity of its effects[2 5 15] and by considerable costs.[4]

Synthetic SMS would appear to have overcome the first of these problems, but still retained the inhibitory activity of its mother peptide. Maximal pentagastrin stimulated acid secretion was effectively and equally suppressed for five hours after a single subcutaneous injection. This was quite unexpected, as in pilot experiments SMS has been estimated to have a plasma half life of around 90 minutes after subcutaneous injection. The data of the above study confirm that the half life of SMS after subcutaneous injection of 100 µg is around 80 minutes. The discrepancy between plasma half life and biological effect is an interesting observation and suggests that plasma concentrations alone cannot be used to monitor the efficacy of the peptide. The plasma data clearly show that the prolonged action of the subcutaneous injection is not because of the slow uptake from the site of injection, but must be related to a direct effect on the SMS receptor.

In an attempt to further characterise the efficacy of SMS, we administered 200 µg of the peptide to the same volunteers in a follow up study. No further inhibition of acid secretion was observed with this dose, despite plasma SMS concentrations which were three times higher than with the lower dose.

Therefore, 100 μg of SMS seem to be a maximal effective subcutaneous dose for suppressing gastric acid secretion. Half life was found to be very similar after both doses and interpatient variability was very low. Therefore nothing will be gained, with regard to acid secretion, by doubling the dose of the peptide.

An interesting observation in these studies is the lack of a significant decrease of acid secretion after a near maximal dose of pentagastrin administered over six hours in the placebo experiments. This suggests that the stomach is capable of secreting near maximal rates for several hours confirming and extending the observations of a recent Scandinavian study in which gastric acid secretion was well maintained for four hours during intravenous pentagastrin doses of 0·1 and 0·5 μg/kg/h.[18] Others have reported a constant secretory rate with doses of pentagastrin ranging from less than 1 to 4 μg/kg/h, but the periods studied did not exceed three hours.[19 20] Wormsley had observed a decrease in acid secretion over a period of three hours in several patients, especially during administration of high doses (6 μg/kg/h) of pentagastrin.[21] He did, however, not use a gastric marker to correct for duodenal volume loss on a regular basis. The effect of somatostatin on acid output was mainly on acid concentration and to a lesser degree on the fluid secretion (Table), confirming our previous results with natural somatostatin.[10 22] It therefore appears that the inhibitory effects of somatostatin peptides is primarily on the quantity of acid secreted and not on the volume.

The ability of the somatostatin analogue to suppress near maximal gastric acid secretion for several hours after subcutaneous injection might render the peptide useful in patients with bleeding peptic ulcers or with peptic ulcer disease not requiring intravenous treatment. None of our volunteers experienced any untoward symptoms at the dose of somatostatin used in the present study. Further studies of the therapeutic potential of this peptide are clearly indicated.

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