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Effect of New Synthetic Analogs of Somatostatin on Gastric Secretion in the Chronic Fistula Dog (39691)

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Somatostatin (growth hormone release inhibiting hormone), a peptide originally isolated from hypothalamic extracts, and the corresponding synthetic tetradecapeptide inhibit growth hormone release *in vitro* and *in vivo* [for review, see Vale *et al.* (1)]. Somatostatin has been shown to inhibit gastric secretion in patients with hypergastrinemia and to reduce basal secretion and food- and exogenous gastrin-stimulated secretion in subjects that were free of gastrointestinal disease (2, 3). Subsequent investigations in dogs and cats have demonstrated that somatostatin inhibits gastric secretion in response both to food and to exogenous gastrin (4–7).

The structural requirements for the antisecretory activity of somatostatin are not known. This report presents data relating to the inhibition of gastric secretion by somatostatin and a number of new synthetic analogs in dogs with a gastric fistula wherein secretion was evoked by pentagastrin.

Methods and materials. Gastric secretion studies were conducted in animals from a colony of 50 female beagle dogs (8–10 kg) having a chronic gastric fistula. Secretion was evoked by the intravenous infusion of a nearly maximal secretory dose of pentagastrin (2.5 μg/kg/hr) for 3 hr (−60–120 min). During the experiment, the fistula was opened and outputs were collected continuously. Samples were taken at 30-min intervals for volume and acid concentration determinations; the latter was obtained by titration to pH 7 with 0.01 *N* NaOH. From these data, total acid output (milliequivalents) was calculated (volume × concentration). Data from placebo trials are reported as the mean ±SE.

Following a 1-hr period required to achieve steady-state secretion during pentagastrin stimulation (−60–0 min), somatostatin or its analogs were infused for 1 hr (0–60 min, 0.77 ml/min). Since there was little difference in their molecular weights, data are reported as the dose administered. The percentage reduction in volume, acid concentration and T.A.O. was determined from the means at each dose level by comparison to a placebo trial in the same animal. The percentage reduction in T.A.O. from each individual animal was used to determine relative potencies and the dose required to reduce output by 80% (ED_{80}, micrograms per kilogram per minute) according to the statistical procedures described by Finney (8).

Pentagastrin (gastrin pentapeptide, blocked) was obtained from Calbiochem, San Diego, Calif. Somatostatin and its analogs were synthetized at the Merck, Sharp and Dohme Laboratories, West Point, Pa. (Table I).

Results. During interdigestive periods, basal gastric acid output in dogs is minimal or absent. In 20 placebo experiments during steady-state secretion, pentagastrin infused at a rate of 2.5 μg/kg/hr produced a near maximal gastric secretory response. In a comparison of two placebo trials in the same animals, there was little variation in the concentration of acid secreted either within or between trials (129 ± 4 at 0 min to 125 ± 5 mequiv/liter at 120 min in trial A versus 133 ± 4 to 127 ± 5 mequiv/liter in trial B). The output volume decreased during the course of the experiment (24 ± 3 ml at 0 min to 16 ± 1 ml at 120 min versus 26 ± 3 to 18 ± 2 ml). T.A.O., the product of volume and concentration, followed the same time course and reflected the same variability as the volume measurements (3.08 ± 0.47 mequiv at 0 min to 2.06 ± 0.19 mequiv at 120 min versus 3.52 ± 0.44 to 2.42 ± 0.24 mequiv).

Somatostatin reduced gastric secretion evoked by pentagastrin. The dose calculated

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TABLE I. PEPTIDE SEQUENCE OF SOMATOSTATIN AND ANALOGS.

Compound	Name	Molecular weight	Sequence
(A)	Somatostatin	1637.6	H—Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys—OH (3, 4, 7, 8, 9, 14)
(B)	Des-(Ala^1,Gly^2)-desamino-Cys^3-somatostatin	1495.7	S—CH_2CH_2CO—Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys—OH
(C)	Des-(Ala^1,Gly^3)-desamino-Cys^3-dicarbasomatostatin	1458.37	CH_2—CH_2—CH_2—CH_2 / CH_2CO—Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser—NH—CH—COOH
(D)	Des-(Ala^1,Gly^2)-desamino-Cys^3-decarboxy-dicarbasomatostatin	1413.6	CH_2—CH_2—CH_2—CH_2 / CH_2CO—Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser—NH—CH_2
(E)	Des-(Ala^1,Gly^2)-desamino-bis-(iNoc)-Lys^{4,9}-dicarbasomatostatin	1728.8	CH_2—CH_2—CH_2—CH_2 / CH_2CO—Lys(iNoc)-Asn-Phe-Phe-Trp-Lys(iNoc)-Thr-Phe-Thr-Ser—NH—CH—COOH
(F)	Bis-*S*-acetamidomethyl-dihydrosomatostatin	1781.7	H—Ala-Gly-Cys(Acm)-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys(Acm)—OH

to reduce T.A.O. by 80% expressed as the ED_{80} (95% C.L.) was 0.06 μg/kg/min (0.02, 0.10). As shown by the data in Table II, somatostatin was about sixfold more effective in reducing the volume of gastric secretion (ED_{80} 0.07 μg/kg/min; 95% C.L., 0.04, 0.11) in comparison to the reduction in acid concentration (ED_{80} 0.41 μg/kg/min; 95% C.L. 0.23, 1.29). There was no significant difference in the potency in the reduction of volume (vol) and T.A.O. between somatostatin and the derivatives des-(Ala^1, Gly^2)-desamino-somatostatin (B), ED_{80} vol = 0.03 (0.01, 0.31). T.A.O. = 0.08 (0.03, 0.23), des-(Ala^1, Gly^2)-desamino-dicarbasomatostatin (C), ED_{80} vol = 0.06 (0.04, 0.14), T.A.O. = 0.04 (0.03, 0.07), and des-(Ala^1, Gly^2)-desamino-decarboxy-dicarbasomatostatin (D), ED_{80} vol = 0.04 (0.03, 0.07), T.A.O. = 0.07 (0.02, 0.17). Although the derivatives cited above (compounds B, C, and D) were equieffective with somatostatin as inhibitors of output volume and T.A.O., they were less effective in reducing the acid concentration. Relative potencies to somatostatin for reduction in acid concentration could not be determined due to differences in the slope of the dose-response lines.

The peak inhibitory effect of somatostatin and the analogs occurred during the latter part of the infusion period (30 to 60 min of collection) and declined after termination of the treatment (30- to 60-min collection period). The duration of action of somatostatin and the analogs, as indicated by activity during the post-treatment period, was short. During the post-treatment period, it was not uncommon for a rebound effect to occur, and outputs exceeded values obtained during the corresponding placebo intervals (see footnote, Table II).

At 8 μg/kg/min, des-(Ala^1, Gly^2)-desamino-bis-(iNoc)-Lys^{4,9}-dicarbasomatostatin (E), an analog having both lysine amino groups protected and bis-*S*-acetamidomethyl-dihydrosomatostatin (F), wherein the cysteine^{3,14} groups were blocked by acetamidomethyl, were essentially ineffective as inhibitors of gastric secretion. No side effects were noted with somatostatin or the analogs.

Discussion. Somatostatin is a potent inhibitor of gastric secretion in both man (2, 3) and experimental animals (4–7). However, the published data indicate only changes in T.A.O. and do not specify if the effect was on output volume or acid concen-

TABLE II. PERCENTAGE CHANGE IN PENTAGASTRIN-EVOKED GASTRIC SECRETION IN CHRONIC FISTULA DOGS[a] (MEAN ± SE)

Compound	Dose[b] (μg/kg/min)	No. dogs	Volume		Acid concentration		Total acid output	
			Peak[c]	Post[d]	Peak[c]	Post[d]	Peak[c]	Post[d]
Placebo	0.0	20	+12 ± 17	± 13 ± 10	+1 ± 4	+5 ± 3	+23 ± 19	+15 ± 12
Somatostatin	0.80	8	−95 ± 2	−76 ± 10	−78 ± 11	−56 ± 11	−99 ± 1	−85 ± 6
(A)	0.20	8	−94 ± 2	−44 ± 18*	−72 ± 12	−33 ± 11*	−97 ± 2	−50 ± 19
	0.08	6	−90 ± 4	−17 ± 31*	−52 ± 6	−28 ± 10*	−94 ± 2	−28 ± 40*
	0.04	7	−81 ± 7	−12 ± 24*	−31 ± 11	−1 ± 12*	−83 ± 8	−2 ± 45*
	0.02	4	−23 ± 17*	+30 ± 31**	−29 ± 13*	−15 ± 7*	−33 ± 21*	+2 ± 21*
	ED_{50}(μg/kg/min)		0.07 (0.04, 0.11)		0.41 (0.23, 1.29)		0.06 (0.02, 0.10)	
(B)	2.40	4	−97 ± 2	−94 ± 4	−54 ± 20	−54 ± 22	−97 ± 2	−98 ± 1
	0.80	4	−96 ± 3	−62 ± 21	−52 ± 19	−40 ± 18*	−97 ± 2	−67 ± 24
	0.20	4	−97 ± 2	−32 ± 20*	−42 ± 20	−26 ± 7	−97 ± 2	−47 ± 17
	0.05	4	−95 ± 2	−35 ± 18*	−47 ± 12	−15 ± 6	−96 ± 2	−43 ± 18*
	0.0125	6	−68 ± 9	−28 ± 18*	−19 ± 21*	−14 ± 6	−68 ± 10	−43 ± 18
	0.006	6	−13 ± 35*	−4 ± 8*	−14 ± 14	−3 ± 4*	−18 ± 36*	−6 ± 9
	ED_{50}(μg/kg/min)		0.03 (0.01, 0.31)				0.08 (0.03, 0.23)	
(C)	0.80	4	−94 ± 1	+1 ± 28*	−63 ± 21	−32 ± 8	−93 ± 3	−36 ± 14
	0.20	4	−90 ± 3	+14 ± 27*	−51 ± 9	−3 ± 9	−96 ± 3	+12 ± 26*
	0.04	4	−63 ± 16	+32 ± 29*	−38 ± 21*	−7 ± 7*	−67 ± 17	+17 ± 18*
	0.01	4	−3 ± 29*	+8 ± 35*	−5 ± 18	−1 ± 10	−2 ± 37*	+15 ± 44*
	ED_{50}(μg/kg/min)		0.06 (0.04, 0.14)				0.04 (0.03, 0.07)	
(D)	0.80	4	−86 ± 10	−60 ± 10	−49 ± 19*	−38 ± 16	−97 ± 2	−62 ± 13
	0.20	4	−91 ± 5	−24 ± 35*	−67 ± 12	−17 ± 5	−96 ± 3	−32 ± 34*
	0.05	4	−83 ± 4	−31 ± 10	−13 ± 10*	−5 ± 7	−88 ± 5	−42 ± 6
	0.025	4	−59 ± 14	−16 ± 29	−14 ± 16*	−24 ± 19*	−59 ± 15	−40 ± 19*
	0.0125	4	+28 ± 91*	+29 ± 15**	−18 ± 5	−6 ± 6	+11 ± 76*	+22 ± 19
	ED_{50}(μg/kg/min)		0.04 (0.03, 0.07)				0.07 (0.12, 0.17)	

[a] Pentagastrin, 2.5 μg/kg/hr (−60 to 120 min).
[b] Dose infused, 0 to 60 min.
[c] Peak effect during treatment (30- to 60-min collection period).
[d] Effect during 30-min post-treatment period (60–90 min).
* Includes values greater than during placebo trial.
** All values greater than during placebo trial.

tration. Reduction in acid concentration would serve to elevate intragastric pH and have potential use in ulcer therapy while reduction of volume alone may not achieve this effect. In the present experiments in the dog, volume was more readily reduced by somatostatin and a sixfold higher dose was required to lower acid concentration.

The inhibitory effects of somatostatin were of short duration and this, along with other biological activities, namely, inhibition of the release of growth hormone, insulin and glucagon (9), may limit its clinical utility in acute hypersecretory states and as an anti-ulcer agent. There is one clinical report of the successful control by somatostatin of a patient with intractable gastrointestinal bleeding. However, during treatment, there was a marked elevation in plasma glucose which responded to administered insulin (10).

In a separate unpublished study in animals from the colony used in the experiments reported herein, somatostatin did not significantly alter plasma glucose, and plasma insulin (radioimmunoassay) decreased from 9 to 3 μU/ml in two of four dogs given somatostatin at 0.8 μg/kg/min for 1 hr. It would therefore appear that adjustment of the dose could allow suppression of gastric secretion without substantially altering blood glucose.

In order to prepare an analog of somatostatin with selectivity for gastric secretion, it was appropriate first to determine some of the structural requirements for antisecretory activity. As with somatostatin, the active analogs were somewhat more potent in reducing volume output than acid concentration. The two amino terminal residues, alanine and glycine, and the remaining terminal group of cysteine were not essential for inhibition of gastric secretion as determined by the effect of des-(Ala[1], Gly[2])-desamino-

somatostatin (B) on T.A.O. It has also been reported that these changes do not alter inhibition of growth hormone release (11). Activity was lost when ring formation was prevented by protection of the thiol groups of the cysteine residues of dihydrosomatostatin (F). This type of modification also caused loss of inhibition of growth and glucagon and insulin release *in vivo* (11).

Analogs locked in a cyclic structure as in des-(Ala^1, Gly^2)-desamino-dicarbasomatostatin (C) and des-(Ala^1 Gly^2)-desamino-decarboxy-dicarbasomatostatin (D) were active. Activity in compound D also indicated that the terminal carboxyl group was not required. However, the importance of the ϵ-amino groups was demonstrated by the loss of activity when they were blocked by isonicotinyloxycarbonyl (compound E).

As with somatostatin, the active analogs (B, C, and D) had a short duration of action. The active analogs differed in that the slope of their dose-response curve for reduction of acid concentration was less steep than that obtained for somatostatin. Since they were less effective in reducing acid concentration, the analogs would also be expected to be less effective in elevating intragastric pH.

Summary. Somatostatin is a potent inhibitor of pentagastrin-evoked gastric secretion in the dog. Exploration of some of the structural requirements for antisecretory activity in this species has revealed that potency in reducing T.A.O. has been retained in compounds wherein alanine (Ala^1) and glycine (Gly^2) and the amino and carboxyl groups of terminal cysteine^{3,14}, have been omitted. Locking the ring structure by replacing the sulfur with carbon retained activity. Compared to somatostatin, the active analogs (des-(Ala^1, Gly^2)-desamino-Cys^3-somatostatin, des-(Ala^1, Gly^2)-desamino-Cys^3-dicarbasomatostatin, and des-(Ala^1, Gly^2)-desamino-Cys^3-decarboxy-dicarbasomatostatin) were slightly less effective in reducing the concentration of acid secreted.

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