Structure-Activity Relationships of Somatostatin Analogs in the Rabbit Ileum and the Rat Colon

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ABSTRACT Somatostatin increases absorption of electrolytes and inhibits diarrhea in patients with endocrine tumors and short bowel syndrome. In an attempt to develop a gut-specific somatostatin analog, each amino acid in the somatostatin molecule was replaced with L-alanine, deleted, or substituted with its D-isomer. The potency of each analog to stimulate ion transport in the rabbit ileum was then determined using the modified Ussing chamber technique. The results were compared to the ability of each analog to inhibit the stimulated release of growth hormone from cultured rat anterior pituitary cells and to inhibit the arginine-stimulated release of insulin and glucagon in the rat in vivo. Analogs that showed gut selectivity were then tested for their ion transport properties in the rat colon. Results: (*a*) Substitution with L-alanine or deletion of the amino acid at position 6, 7, 8, or 9 and deletion of Threonine^{10}-produced analogs with significantly reduced ion transport properties to <4% of somatostatin's action. The substitution also markedly reduced the ability of the compounds to inhibit the release of growth hormone, insulin, and glucagon. (*b*) Selectivity of intestinal ion transport was achieved by any one of the following alterations: L-alanine substitution at Phenylalanine^{11}, deletion of Phenylalanine^{11}, substitution with D-lysine at Lysine^{4}, or substitution with L-alanine at Lysine^{4}. These compounds had intestinal ion transport properties of 52, 34, 139, and 94%, respectively, while demonstrating little or no inhibition of growth hormone, insulin or glucagon release. Conclusions: (*a*) Phenylalanine^{6}, Phenylalanine^{7}, Tryptophan^{8}, and Lysine^{9} are required for the ion transport and other biologic actions of somatostatin, whereas Threonine^{10} serves as an essential spacer. (*b*) Alteration at Phenylalanine^{11} or Lysine^{4} yields analogs that are selective for ion transport in the rabbit ileum and rat colon. These findings should be taken into consideration when developing a gut-specific somatostatin analog that can be useful in the treatment of diarrhea.

INTRODUCTION

Somatostatin, a tetradecapeptide found in the D cells and neural tissue throughout the gastrointestinal tract and pancreas, has several actions suggesting that it can be useful in the treatment of diarrheal syndromes. Somatostatin (*a*) stimulates sodium and chloride absorption in the rabbit ileum in vitro (1, 2); (*b*) inhibits secretagogue-induced water and electrolyte secretion in the rat jejunum in vivo and rat colon in vitro (3–5); (*c*) decreases intestinal motility (6, 7); (*d*) inhibits the release of hormones or neurotransmitters that may contribute to diarrhea, e.g., vasoactive intestinal polypeptide, gastric inhibitory polypeptide, gastrin, and secretin (8, 9); and (*e*) inhibits diarrhea in patients with carcinoid tumors (10, 11) and short bowel syndrome (12). Since somatostatin affects many other organ systems (8), its present use in any one specific clinical situation is limited. Therefore, the development of a gut-specific somatostatin analog may allow it to be utilized as a therapeutic agent in the treatment of diarrhea. This report describes our attempt to develop a somatostatin analog with fewer side effects. The biologic effects of each analog were tested to determine what aspects of the somatostatin molecule are gut-specific.

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We synthesized and tested three series of somatostatin analogs, in which each of the 14 amino acids of somatostatin were (*a*) replaced with L-alanine, (*b*) deleted, or (*c*) substituted with its D-isomer. In the alanine series, individual amino acids were systematically replaced with L-alanine. Replacement with L-alanine allowed us to evaluate the importance of the functional group of each amino acid for biologic activity, since the original backbone conformation of somatostatin is maintained with such analogs. In the des-amino acid series, individual amino acids were successively deleted from the somatostatin molecule. The results of this series allowed us to evaluate the importance of the position of each amino acid for the biologic activity of somatostatin. Finally, in the D-amino acid substitution series, each amino acid was replaced with its D-isomer, thus changing the backbone conformation of the molecule. This series was of particular interest because of the dissociation of biologic activities that certain analogs exhibited in other systems (13–18).

METHODS

All peptides were synthesized in our laboratory by solid phase methodology, according to the method of Rivier et al. (19, 20). The potency of each somatostatin analog was determined in isolated rabbit ileum, using the modified Ussing chamber technique, as described by Binder et al. (21). New Zealand white male rabbits weighing 2–3 kg were killed with an intravenous injection of 50 ml air bolus and the distal ileum removed. After rinsing, isolated segments of mucosa stripped of the serosa and part of the muscular layer were mounted between two lucite chambers with a surface area of 1.13 cm^2. Both sides of the tissue were bathed with Ringer solution, pH 7.4, at 37°C and bubbled continuously with 95% O_2 and 5% CO_2. The Ringer solution contained (in millimolars): Na, 140; K, 5.0; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO_3, 25; HPO_4, 2.4; and H_2PO_4, 0.4. 10 mM glucose was present in the serosal bathing media and 10 mM mannitol was present in the mucosal bathing media. In rat experiments, nonfasting male Sprague-Dawley rats weighing 250–300 g were killed with ether anesthesia. The colon was rapidly removed and rinsed with iced Ringer's solution. The muscular layers were then removed and the tissue was mounted, as described for the rabbit ileum. 10 mM glucose was present in both the serosal and the mucosal bathing media.

The potential difference (PD)[1] across the mucosa was measured by calomel half-cell electrodes in 3 M KCl and monitored with a potentiometer. The spontaneous tissue PD was short-circuited and nullified by an automatic voltage clamp with Ag:$AgCl_2$ electrodes throughout the experiment, except for 5–10 s every 5 min when the PD was recorded. Tissue conductance (G) was calculated from the PD and the short-circuit current (I_{sc}) according to Ohm's law. In experiments with rabbit ileum, each analog was added to the serosal reservoir of the Ussing chamber at 50 min. The change in I_{sc} was calculated as the difference in I_{sc} between 65 and 50 min. In rat experiments, each analog was added to the serosal reservoir at 30 min, and the change in I_{sc} calculated as the difference between 45 and 30 min. At each dose, the change in I_{sc} represents the mean of 3 to 11 determinations using separate tissue samples for each measurement. At least three concentrations of the analog were tested against somatostatin. To limit the possibility of tachyphylaxis, no repetitious additions were made to the same tissue.

Calculation of the relative potency. All relative potency calculations were carried out on the Salk Institute VAX-11 computer with the computer program BIOPROG, written by Vivian B. Faden and David Rodbard, M.D. of the Biophysical Endocrinology Section, Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205.

The relative potency was calculated by conducting a parallel line type of analysis in which the dose-response curve of the somatostatin analog is compared with the dose-response curve of somatostatin. This type of analysis is based on the analysis of bioassay data as described by Finney (22). The dose response curves are linear and are tested for nonparallelism by a *t* test. If there is significant nonparallelism then this type of analysis is invalid. In this study, nonparallelism is caused by a lack of biologic activity of the analog tested. The lines are constrained to be parallel and the relative potency is then calculated from the difference in dosage required to cause an equivalent effect and is expressed as a percentage of the potency of somatostatin. The upper and lower 95% confidence limits for the potency estimate are then determined. If the upper 95% confidence limit is <100, i.e., the potency assigned somatostatin, then the analog is significantly less potent than somatostatin. Conversely, if the lower limit is greater than 100, then the analog is significantly more potent than somatostatin.

The relative potency for intestinal ion transport obtained was compared to the inhibition of the stimulated release of growth hormone from cultured rat anterior pituitary cells in vitro and inhibition of arginine-stimulated insulin and glucagon release in the rat in vivo; the majority of these results have been previously published (13–18).

RESULTS

Validity of I_{sc} as measure of ion flux. Unidirectional chloride fluxes were determined for rabbit ileum, using ^{36}Cl oppositely directed on adjacent pieces of tissues. This method permitted the characterization of bidirectional fluxes under short-circuited conditions. Therefore, we can determine the changes of ion flux, as well as changes in electrical potential difference and I_{sc} caused by addition of a somatostatin analog. Fig. 1 demonstrates the correlation between the change of net chloride flux and the change of the I_{sc} affected by somatostatin and selected somatostatin analogs. A linear correlation between the change of the I_{sc} and the change of net chloride flux was demonstrated with an *r* value of 0.93, $P < 0.001$. Because the change of I_{sc} was directly related to the change of ion flux, the I_{sc} was used to construct the graded dose response curves from which the relative potency of each somatostatin analog was calculated. Examples of

[1] *Abbreviations used in this paper*: I_{sc}, short-circuit current; PD, potential difference.

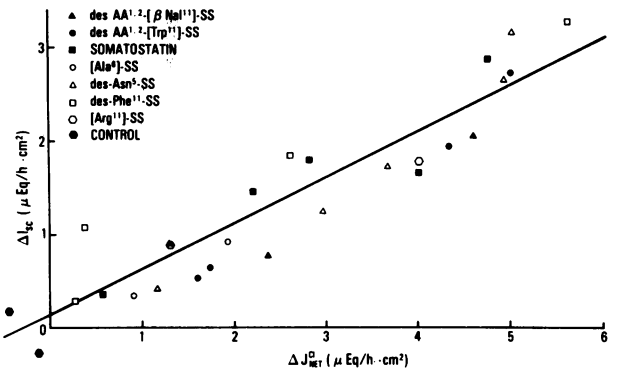

FIGURE 1 Linear correlation between the change of I_{sc} on the vertical axis and the change of net chloride flux (J_{net}) on the horizontal axis for somatostatin and selected analogs at concentrations of 10^{-6} to 10^{-8} M in the rabbit ileum. The I_{sc} is passed by the automatic voltage clamping apparatus to nullify the potential difference across the isolated intestine. I_{sc} is used in this study as an indicator for alteration of ion transport. The changes of net chloride flux were calculated from alteration of chloride movement in both directions (lumen side to blood stream side and blood stream side to lumen side) using radioactive tracer, as described in Methods. $r = 0.93$, $P < 0.001$, $n = 27$.

the graded dose effect of somatostatin and some analogs are shown in Fig. 2.

Relative potencies of somatostatin analogs

Three series of somatostatin analogs were synthesized and tested in the rabbit ileum. The alanine series allowed us to evaluate the role of the functional groups of the individual amino acids, the des-amino acid series to evaluate the importance of the position that each

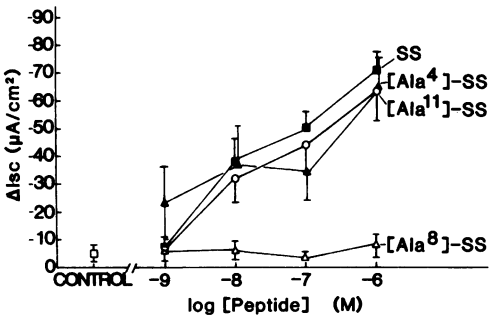

FIGURE 2 Graded dose effects of Ala[4]-somatostatin, Ala[8]-somatostatin, and Ala[11]-somatostatin on the changes in the I_{sc}, as compared to somatostatin, are shown. At each dose, the change in I_{sc} represents the mean of four to eight determinations, using separate tissue samples for each measurement. To exclude the possibility of tachyphylaxis, no repetitious additions were made to the same tissue. The relative potency of each analog is calculated from its dose-response curve as compared with somatostatin, as described in Methods.

amino acid has in the peptide chain, and the D-amino acid series to study the importance of the backbone conformation for biologic activity. These results led us to synthesize and test analogs with multiple deletions or alterations at specific positions. Selected analogs that showed gut selectivity were then tested in the rat colon to ascertain that the different structure-activities requirement is not a result of species differences.

Alanine series. Table I shows the relative potencies of the analogs in this series on the release inhibition of hormones and on ion transport. Alanine substitution for glycine (Gly[2]), lysine (Lys[4]), asparagine (Asn[5]), threonine (Thr[10]), phenylalanine (Phe[11]), Thr[12], and serine (Ser[13]) did not change the intestinal ion transport activity significantly, suggesting that these functional groups are expendable. Replacement of Phe[6], Phe[7], tryptophan (Trp[8]), or Lys[9] results in analogs with little or no biological activity for ion transport (up to 10^{-6} M). The results for intestinal ion transport in the rabbit ileum are similar to those for the inhibition of growth hormone, insulin and glucagon release in the rat. However, there are two exceptions. (*a*) L-alanine substitution for phenylalanine at position 11 yields an analog with markedly reduced activity to inhibit growth hormone, insulin, and glucagon release in the rat, but with significant ion transport activity in both the rabbit ileum (Table I) and the rat colon (Table VI). (*b*) Substitution of Lys at position 4 by L-alanine resulted in dissociation of biologic activities favoring intestinal ion transport and inhibition of growth hormone release.

des-amino acid series. The results of this series are summarized in Table II. Removal of the linear portion of the molecule, Ala[1] or Ala[1]-Gly[2], caused no reduction in any of the biologic activities studied, indicating that this portion of the peptide is expendable. However, deletion of any single amino acid from the 12-membered ring resulted in analogs with reduced biologic activities. The deletion of a single amino acid at Phe[6], Phe[7], Trp[8], Lys[9], or Thr[10] resulted in analogs without intestinal ion transport potency. These results, however, together with those obtained from the alanine substitution series, confirmed that in the intestine, as in other endocrine systems, the sequence from position 6 to 9 is critical for biologic activity and probably for receptor binding or activation. The significant reduction of the ion transport property when Thr[10] was deleted, but not when it was replaced by alanine, suggests that the 10th amino acid is an essential spacer, i.e., an amino acid is needed to fill this space or position but is not required to be threonine. The removal of Phe[11] from the somatostatin molecule yielded an analog with intestinal ion transport activity, confirming that Phe[11] is not critical for ion transport in the rabbit ileum.

TABLE I

Relative Potencies of Alanine-substituted Somatostatin Analogs

Peptide	I_{sc} Induced by the analog at				Relative potencies			
					Rabbit ileal ion transport	Release inhibition of rat hormones		
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M		Insulin	Glucagon	Growth hormone
Somatostatin (SS)					100	100	100	100
[Ala^2]-SS (SS1)*	14±5	23±7	40±8	54±11	179 (28–1530)‡	135	279	180
[Ala^4]-SS (SS3)	23±13	38±13	34±10	65±10	94 (20–392)	12	12	60
[Ala^5]-SS (SS2)	5±4	27±6	47±2	43±10	135 (30–550)	132	176	130
[Ala^6]-SS (SS2)	7±1	1±5	20±6	22±6	4	<10	<10	<1
[Ala^7]-SS (SS2)	12±8	4±3	2±2	23±6	4	<10	<10	3
[Ala^8]-SS (SS2)	7±5	6±4	4±2	13±5	<1	<10	<10	<1
[Ala^9]-SS (SS2)	5±3	7±1	5±1	9±4	<1	<1	<10	<1
[Ala^{10}]-SS (SS2)	4±1	13±2	24±10	38±9	26 (4–136)	14	<10	100
[Ala^{11}]-SS (SS3)	16±5	36±9	51±12	71±11	52 (6–373)	<10	<10	3
[Ala^{12}]-SS (SS2)	3±1	10±1	39±15	58±13	105 (24–449)	26	ND§	100
[Ala^{13}]-SS (SS2)	18±9	14±5	33±11	58±16	122 (24–576)	27	<10	250
Control: SS1	16±5	21±5	30±5	55±7				
SS2	7±1	18±2	36±4	56±4				
SS3	14±4	45±10	57±9	76±8				

The values of I_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of three to nine animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list. The relative potencies of each analog for its biological function were determined by comparing the graded dose effect of the analog to the appropriate somatostatin control. In the rabbit ileal experiment, the concentration of each compound tested ranged from 10^{-9} M to 10^{-6} M. For each analog, three to nine separate tissues from different animals were tested at each concentration without repetitious additions. The relative potencies are expressed as mean values.

‡ Values in parentheses represents the range of potency, as compared to somatostatin, that covers 95% of all experiments done based on each individual piece of intestine. Ranges are not given for (Ala^6, Ala^7, Ala^8, or Ala^9)-SS, because the lack of potency makes the dose-response curve significantly nonparallel in comparison with the somatostatin curve, and therefore, the range obtained is not valid.

§ Not determined.

Therefore, deletion or alteration of Phe^{11} may result in gut-selectivity.

D-Amino acid substitution series. Data in Table III indicate that D-Lys^4-somatostatin has a large dissociation of biologic activities favoring intestinal ion transport and growth hormone release inhibition. Insulin and glucagon release was not affected by D-Lys^4-somatostatin. D-cysteine (Cys^3)-, and D-Cys^{14}-somatostatin exhibited some dissociation of the biological activities. Both D-Phe^6- and D-Ser^{13}-somatostatin though significantly less potent than somatostatin, retained some biologic activity and selectivity for intestinal ion transport. D-Trp^8-somatostatin, which is more potent than somatostatin for inhibition of growth hormone, insulin, and glucagon release, was not significantly more potent than somatostatin in the rabbit ileum. When tested in rat colon, D-Lys^4-, D-Phe^6-, and D-Ser^{13}-somatostatin demonstrated relative potencies similar to those observed in rabbit ileum (Table VI).

Multiple deletion. It is possible that a peptide containing the amino acid sequence from positions 6 to 10, with either deletion or an appropriate substitution for Phe^{11} may be selective for intestinal ion transport. Therefore, we synthesized and tested somatostatin analogs that contained the critical amino acid sequence from positions 6 to 10 of the somatostatin molecule together with one or more spacer amino acids and the disulfide bridge provided by two cysteine residues. The activities of these analogs are summarized in Table IV. As in other systems, the potency of the analog fell with deletion of more than 6 amino acids (17). None of the compounds tested showed significant selectivity for intestinal ion transport.

Substitution at Phe^{11}. Since phenylalanine at position 11 appeared to be critical for the inhibition of growth hormone, insulin, and glucagon release, but not for intestinal ion transport, we tried to enhance this dissociation of biologic activities by substituting

TABLE II

Relative Potencies of Single Amino Acid-deleted Somatostatin Analogs

Peptide	I_{sc} Induced by the analog at 10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	Relative potencies: Rabbit ileal ion transport	Release inhibition of rat hormones: Insulin	Glucagon	Growth hormone
Somatostatin (SS)					100	100	100	100
des-Ala^1-SS (SS1)*	ND‡	10±2	41±8	56±11	63 (8–369)§	ND	ND	ND
des-Ala^1,Gly^2-SS SS2)	ND	22±2	44±3	49±5	231 (31–3013)	61	ND	60
des-Lys^4-SS (SS3)	ND	12±2	27±4	47±8	6 (0.6–23)	1.5	0	3
des-Asn^5-SS (SS4)	ND	38±8	49±7	58±9	11 (1–117)	10	<1	4
des-Phe^{6}SS (SS5) (=des-Phe^7-SS)	ND	11±1	5±6	22±5	3	<1	ND	<1
des-Trp^8-SS (SS6)	9±1	8±1	4±3	12±5	<1	<1	<1	<1
des-Lys^9-SS (SS5)	ND	6±2	−2±1	6±2	<1	ND	ND	<1
des-Thr^{10}-SS (SS5)	ND	6±0	2±2	13±6	<1	<1	<1	<1
des-Phe^{11}-SS (SS7)	1±8	11±3	35±18	75±14	34 (12–90)	<1	<1	2
des-Thr^{12}-SS (SS5)	ND	9±0	27±10	36±4	21 (3–124)	<1	<1	2
des-Ser^{13}-SS (SS5)	ND	13±4	18±11	26±7	9 (1–59)	<1	<1	2
Control: SS1	ND	16±4	44±9	61±15				
SS2	ND	25±6	37±6	42±5				
SS3	ND	31±3	53±8	70±8				
SS4	ND	44±7	61±8	69±6				
SS5	7±1	18±2	36±4	56±4				
SS6	20±3	75±6	82±4	89±12				
SS7	2±7	28±9	47±9	89±20				

The values of I_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of three to nine animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list.

‡ Not determined.

§ 95% confidence limits are shown in parentheses. Significant nonparallelism of the dose response curve occurred with des-Phe^6, Trp^8, Lys^9, or Thr^{10}-SS.

different amino acids for Phe^{11}. Table V shows the relative potencies of these analogs. The biologic activity in the intestine did not change with replacement by a neutral or basic amino acid, but decreased with the acidic amino acid, glutamic acid. The dissociation of biologic activities was most apparent with Ala^{11}- and histidine (His^{11})-somatostatin. The halogenated derivatives of phenylalanine were active in the intestine, but were not gut-specific. des-AA^{1,2,4,5,12}-[D-Trp^8,Trp^{11}]-somatostatin, which contains the critical sequence of amino acid from position 6 to 10 and an alteration of position 11, was biologically active in the rabbit and rat intestine.

DISCUSSION

This study clearly demonstrates that the amino acids at positions 6 to 10 of somatostatin are important for stimulation of intestinal ion transport. These amino acids appear to be necessary for expression of all the

TABLE III

Relative Potencies of D-Amino Acid-substituted Somatostatin Analogs

Peptide	I_{sc} Induced by the analog at				Relative potencies			
						Release inhibition of rat hormones		
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	Rabbit ileal ion transport	Insulin	Glucagon	Growth hormone
Somatostatin (SS)					100	100	100	100
[D-Ala[1]]-SS (SS1)*	ND‡	32±8	57±13	58±4	105 (25–530)§	69	91	ND
[D-Cys[3]]-SS (SS1)	ND	23±7	54±11	74±13	103 (26–498)	7	<10	50
[D-Lys[4]]-SS (SS2)	ND	14±3	32±5	65±9	139 (23–886)	1	1	22
[D-Phe[6]]-SS (SS1)	ND	23±5	21±5	53±9	10 (3–40)	<1	<1	5
[D-Phe[7]]-SS (SS1)	ND	15±4	11±3	26±4	1 (0.2–6)	<3	<3	<1
[D-Trp[8]]-SS (SS1)	18±7	37±7	51±8	43±10	256 (63–1,139)	850	650	800
[D-Lys[9]]-SS (SS1)	ND	16±5	11±3	19±10	1 (0.1–3)	<1	<1	<1
[D-Thr[10]]-SS (SS3)	ND	7±4	16±4	39±6	5 (1–40)	ND	ND	<1
[D-Phe[11]]-SS (SS1)	−6±2	4±4	18±1	33±5	3 (0.4–11)	<1	<1	10
[D-Thr[12]]-SS (SS1)	ND	17±3	29±8	64±13	20 (4–101)	ND	ND	20
[D-Ser[13]]-SS (SS1)	ND	19±4	36±9	46±10	13 (3–56)	5	<1	10
[D-Cys[14]]-SS (SS4)	−2±1	38±6	48±11	57±12	336 (38–6,800)	20	310	270
Control: SS1	16±5	36±9	51±12	71±11				
SS2	ND	25±4	41±7	45±6				
SS3	ND	25±4	40±7	47±6				
SS4	−2±0	28±5	40±11	51±11				

The values of I_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of 3 to 11 animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list.

‡ 95% confidence limits are shown in parentheses.

§ Not determined.

biologic activities of somatostatin (17–22). Receptor binding studies also point to the importance of the amino acids at positions 6 to 10; alteration of this region results in a marked reduction of receptor affinity (23). We believe that the critical region of somatostatin consists of the amino acids at positions 6, 7, 8, and 9, with their functional groups involved in receptor binding and activation. Substitution with L-alanine, deletion, or replacement with the D-isomer for Phe[6], Phe[7], Trp[8], or Lys[9] produces analogs with essentially no ion transport properties and with no ability to inhibit the release of growth hormone, insulin, and glucagon. An exception is the D-Trp[8]-somatostatin analog. This analog retains intestinal ion potency, and has an eightfold more potent inhibition of growth hormone, insulin, and glucagon release than does somatostatin. The high potency of D-Trp[8]-somatostatin may reflect the stabilization of a turn by the D-isomer (24, 25). We believe that Thr[10] acts as an important spacer because deletion of Thr[10] reduces all biologic activities to near zero, but activity is retained when alanine is substituted for Thr[10].

The conformational constraint of the critical region, i.e., amino acids 6 to 9, may be quite important for receptor recognition. One of the key determinants of the orientation of the critical region is the disulfide bond created by the cysteine residues at positions 3 and 14 of somatostatin (20). It may be possible to retain biologic activities in analogs containing only these critical amino acids, provided the appropriate bridging units are used. Veber et al. (26) have synthesized stable bicyclic analogs of somatostatin containing the amino acids from positions 7 to 10 which are biologically active (26, 27). In addition, one of the bicyclic analogs, cyclo(7-aminoheptanoic acid-Cys-Phe-D-Trp-Lys-Thr-Cys), is held in the conformation required by use of aminoheptanoic acid that serves as a second ring. It is clear from the potency of these analogs, which is equal or greater than somatostatin for the inhibition of growth hormone, insulin and glucagon release, that the elements necessary for expression of biologic activity are the amino acids Phe-D-Trp-Lys-Thr and a bridging unit to hold them in the correct conformational constraint.

It is interesting to see that alterations of the amino acids adjacent to the critical region result in the se-

TABLE IV

Relative Potencies of Somatostatin Analogs with Multiple Deletions

Peptide	I_{sc} Induced by the analog at				Relative potencies			
						Release inhibition of rat hormones		
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	Rabbit ileal ion transport	Insulin	Glucagon	Growth hormone
Somatostatin (SS)					100	100	100	100
des-Asn^5-[D-Trp^8, D-Ser^{13}]-SS (SS1)*	11±3	33±8	82±2	87±21	25 (5–189)‡	1750	<1	13
des-AA^{1,2,4,5,12} [D-Trp^8]-SS (SS2)	ND§	10±3	49±6	69±7	257 (52–1483)	70	20	45
des-AA^{1,2,4,5,12,13} [D-Trp^8]-SS (SS1)	6±7	25±8	65±14	67±12	5 (0.1–48)	4	45	7
des-AA^{1,2,4,5,10,12,13} [D-Trp^8]-SS (SS2)	ND	ND	8±1	14±3	1 (0.2–6)	<0.01	<1	<1
des-AA^{1,2,4,5,11,12,13} [D-Trp^8]-SS (SS3)	ND	4±5	10±6	17±2	<1	0.07	<1	<1
Control: SS1	20±4	75±13	82±8	89±21				
SS2	7±1	17±2	36±4	56±4				
SS3	ND	42±5	46±8	58±15				

The values of I_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of three to seven animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list.

‡ 95% confidence limits are shown in parentheses.

§ Not determined.

lectivity of the biological actions. The dissociation of biologic activities may be the result of slight changes in the conformation of the critical region caused by these amino acids. In this study, the importance of Phe^{11} and Lys^4 for gut selectivity has been demonstrated. Previous results indicate that deletion of Asp^5 results in selective insulin inhibition (28, 29).

Phenylalanine at position 11 is required for inhibition of growth hormone, insulin and glucagon release but is not necessary for intestinal ion transport. Substitution with alanine for Phe^{11} in the somatostatin molecule allows the analog to selectively retain the biologic potency of 52% in the intestine. There was a striking difference between the potency in the gut and other biological systems tested, all of which were <10%. Also, deletion of Phe^{11} yields an analog that retains significant and selective ion transport activity of 34%, as compared with 2% or less of other biological activities tested. Substitution of Phe^{11} with other amino acids also exhibits selectivity for intestinal ion transport (Table VI). This finding suggests that the aromatic ring of Phe^{11} is not an absolute requirement for intestinal ion transport activity. The only amino acid substitution that causes a significant reduction in potency is glutamic acid. Glutamic acid substitution makes the analog less soluble. Further evidence that Phe^{11} is not critical for intestinal activity is suggested by the halogenated derivatives of Phe^{11}. -Cl-Phe^{11}- and I-Phe^{11}-somatostatin are analogs with modification of the aromatic ring. They are eight- to ninefold more potent than somatostatin for inhibiting the release of insulin and glucagon, but intestinal ion transport potency is not significantly higher. The results suggest that a gut-specific somatostatin analog may be obtained by suitable substitution at position 11.

Replacement for lysine at position 4 with alanine or the D-isomer produces analogs that favor intestinal ion transport and inhibition of growth hormone release. Murphy et al. (30, 31) have shown that alteration of Lys^4 results in analogs with selective inhibition of growth hormone release. The importance of Lys^4 seems to be shared by both intestinal ion transport properties and inhibition of the release of growth hormones. The lack of adverse effects of long-term growth hormone deficiency in adults increases the possibility that with alteration at Lys^4 these analogs may be useful clinically as antidiarrheal agents.

From the results obtained with their analogs, Veber et al. (26, 27) have theorized that Phe^6 and Phe^{11} stabilize the biologically active conformation of the peptide through hydrophobic bonding of their aromatic rings and that the phenylalanines are not involved with

TABLE V

Relative Potencies of Somatostatin Analogs with Substitutions at Phenylalanine[11]

Peptide	I_{sc} Induced by the analog at				Relative potencies			
						Release inhibition of rat hormones		
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	Rabbit ileal ion transport	Insulin	Glucagon	Growth hormone
Somatostatin (SS)					100	100	100	100
[Ala[11]]-SS (SS1)*	16±5	36±9	51±12	71±11	52 (6–373)*	<10	<10	3
[His[11]]-SS (SS2)	7±3	7±2	26±5	53±9	48 (9–209)	<1	<1	3
[Arg[11]]-SS (SS2)	5±5	19±3	42±8	83±13	291 (82–1,264)	142	<1	2
[Trp[11]]-SS (SS3)	6±4	31±9	52±9	82±5	249 (84–855)	ND§	ND	22
[Glu[11]]-SS (SS2)	−8±1	13±4	11±1	25±12	9 (1–44)	<1	<1	<0.1
des-AA[1,2]-[Trp[11]]-SS (SS2)	ND	24±3	54±3	68±3	405 (82–2,322)	ND	ND	53
des-AA[1,2]-[−Nal[11]]-SS (SS2)	ND	12±2	49±5	49±10	107 (21–543)	ND	ND	190
des-AA[1,2,4,5,12][D-Trp[8], Trp[11]]-SS (SS4)	ND	4±5	10±6	17±2	37 (2–328)	ND	ND	18
[−Cl-Phe[11]]-SS (SS2)	0±3	16±6	42±3	53±6	100 (23–389)	800	900	78
[−I-Phe[11]]-SS (SS2)	−5±8	18±9	27±7	48±14	44 (8–194)	950	900	100
[*N*-Me-Phe[11]]-SS (SS2)	7±4	11±4	9±8	54±6	25 (4–119)	13	<10	ND
Control: SS1	14±4	45±10	57±9	76±8				
SS2	7±1	18±2	36±4	56±4				
SS3	5±2	18±6	38±7	77±13				
SS4	ND	42±5	46±13	58±14				

The values of I_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of three to eight animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list.

‡ 95% confidence limits are shown in parentheses.

§ Not determined.

receptor binding. Their theory is in agreement with the observations that the phenylalanines at positions 7 and 11 are required for growth hormone, insulin, and glucagon inhibition, and that halogenated derivatives of Phe[11] have equal or greater potency than somatostatin. However, since substitution at Phe[11] with nonaromatic and nonhydrophobic amino acids such as histidine and arginine yield analogs with significant intestinal ion transport, creation of hydrophobic bonding appears not to be critical for biologic activity in the intestine. The importance of Phe[6] for intestinal ion transport may lie with the direct interaction of the aromatic ring with the receptor or Phe[6] may maintain the correct conformation of the critical region without interaction with Phe[11].

All our analogs with multiple deletions were held in cyclic forms with the cysteine rings. We found that deletion of more than six amino acids results in analogs with virtually no biologic activity, including analogs which contain all the critical amino acids (Table IV, 17). This is probably due to the loss of the conformation required for receptor binding and activation.

Data on the biologic activity of an analog relative to somatostatin should be interpreted with caution. Structure-activity studies do not differentiate variations in (*a*) affinity of the receptor for the analog, (*b*) the ability of the receptor/peptide complex to induce appropriate intracellular signals, or (*c*) the metabolism of the peptide. Further care must be taken in the interpretation of selectivity. Our study with analogs that stimulate ion transport in the rabbit ileum and rat colon suggests that intestinal somatostatin receptors in these two species are similar. It is not known whether somatostatin receptors are the same in different mammals. Since, no variation has been found in the amino acid sequence of native somatostatin-14 in the mammals studied, sheep, pig, and rat (32–35), it is likely that receptors for somatostatin-14 are similar. Recently, the existence of multiple forms of somatostatin and its precursors besides somatostatin-14 has been demonstrated in angler fish (36, 37). This raises the possibility of the presence of multiple somatostatins and somatostatin receptor subtypes similar to the opioid peptides, for example, morphiceptin, enke-

TABLE VI

Relative Potencies of Selected Somatostatin Analogs for Ion Transport in the Rat Colon

Peptide	I_{sc} Induced by the analog at					Relative potency
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	Intestinal ion transport
Somatostatin (SS)						100
Alanine substituted analog						
[Ala^{11}]-SS (SS1)*	ND‡	21±4	34±6	57±15	ND	27 (2–160)§
Single amino acid-deleted analogs						
des-Lys^{4}-SS (SS2)	ND	25±11	29±4	33±6	61±10	3 (0.3–21)
des-Asn^{5}-SS (SS2)	ND	17±1	23±5	47±14	69±13	7 (0.8–48)
des-Phe^{11}-SS (SS3)	ND	11±8	39±5	46±10	ND	9 (0.3–58)
des-Thr^{12}-SS (SS3)	ND	18±6	39±8	37±8	ND	5 (0–52)
D-Amino acid substituted analogs						
[D-Lys^{4}]-SS (SS2)	ND	39±9	38±7	54±15	ND	100 (6–2247)
[D-Phe^{6}]-SS (SS2)	ND	21±4	26±4	56±13	ND	10 (0.7–64)
[D-Ser^{13}]-SS (SS2)	ND	27±5	34±15	57±15	ND	24 (1–232)
Multiple deletions with alteration at Phe^{11}						
des-AA^{1,2,4,5,12}-[D-Trp^{8}, Trp^{11}]-SS (SS2)	19±8	32±6	57±10	62±16	ND	113 (11–1211)
Alteration at Lys^{4}						
[−NH$_2$-Phe^{4},D-Trp^{8}]-SS (SS4)	ND	36±11	60±11	73±5	ND	89 (8–864)
Control: SS1	ND	25±4	51±4	62±16	ND	
SS2	15±5	30±4	41±5	60±10	ND	
SS3	ND	22±8	66±12	77±12	ND	
SS4	ND	39±5	62±8	70±7	ND	

The values of T_{sc} represent the mean±SE in $\mu A/1.13$ cm^2 of four to eight animals.

* The appropriate somatostatin control for each analog is indicated in parentheses following the name of the analog. The result of each SS control is included at the end of the list.

§ 95% confidence limits are shown in parentheses.

‡ Not determined.

phalin and dynorphin for mu, delta, and kappa receptors, respectively. It remains to be proven whether the selective analogs identified in this study will show gut selectivity in man.

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