Radioimmunoassay of Human Plasma Somatostatin

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SUMMARY

Human plasma somatostatin-like immunoreactivity (SLI) is heterogeneous. Moreover, human plasma contains a somatostatin-binding protein, enzymes that degrade the peptide, and other poorly characterized substances that interfere in radioimmunoassays of the peptide. Previously described extraction methods only partially circumvent these problems and generally result in less than adequate recovery. The present studies were therefore undertaken to determine whether gel filtration of plasma could be used to avoid these difficulties and provide a means for radioimmunoassay of plasma somatostatin in man. Gel filtration (Sephadex G25) of human plasma and subsequent radioimmunoassay of column eluates using five different antisera uniformly demonstrated two fractions of SLI in plasma. One fraction (peak I) eluted in the void volume and had an apparent molecular weight in excess of 150,000 daltons; this material comprised most of the plasma SLI, did not dilute in parallel with synthetic somatostatin, and decreased immunoprecipitability of both N-tyrosine- and tyrosine-11-^{125}I somatostatin. The second fraction (peak II) coeluted with synthetic somatostatin (apparent mol. wt. 1600 daltons), diluted in parallel with synthetic somatostatin, and did not appreciably degrade either ^{125}I-tyrosylated analogue. Synthetic somatostatin added to plasma was recovered nearly completely (95 ± 3%) in peak II SLI. Infused synthetic somatostatin increased peak II SLI but did not alter peak I SLI. These results demonstrate that gel chromatography of human plasma before assay can be used to circumvent problems of immunoheterogeneity, degradation, and nonspecific interference while providing adequate recovery. With this method, mean plasma somatostatin concentrations (peak II SLI) in postabsorptive normal volunteers ranged from approximately 40 to 100 pg/ml, depending on the antibody system used. These concentrations (3–6 × 10^{-11} M) are of similar magnitude to those reported to affect reputed target tissues of somatostatin in vitro and are thus consistent with a possible hormonal function for the peptide. DIABETES *30*:728–734, September 1981.

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Radioimmunoassay of somatostatin in human plasma is subject to numerous difficulties: unextracted plasma can degrade both native somatostatin and tyrosylated analogues used as tracers in the assay.[1–3] Moreover, plasma contains a somatostatin-binding protein[4,5] and other as yet poorly characterized substances that interfere with antibody binding.[6–8] Finally, plasma somatostatin-like immunoreactivity (SLI) is heterogeneous with only a small proportion of the plasma SLI being attributable to a 1600-dalton molecule corresponding to somatostatin.[2,9]

Current methods of extracting human plasma to avoid tracer degradation and interference with antibody binding do not result in complete recovery of somatostatin from plasma.[6–10] Furthermore, the SLI extracted from rat plasma by methods similar to those used for human plasma is still heterogeneous.[11–14] The present studies were therefore undertaken to determine whether gel filtration of human plasma could be used to circumvent the above difficulties. Our results indicate that gel filtration of plasma before radioimmunoassay alleviates degradation of trace and interference with antibody binding, provides near complete recovery of somatostatin from plasma, and permits determination of the SLI in plasma due to the 1600-dalton molecule.

METHODS

Collection of specimens. Blood (10 ml) was collected from overnight-fasted normal subjects into chilled tubes (Kimble-Terumo, Elkton, Maryland) containing EDTA (11 mg)-potassium sorbate (0.015 mg) and immediately centrifuged for 5 min at 2000 × *g* at 4°C. The resultant plasma was either stored at −20°C for further studies or immediately subjected to gel filtration (see below).

Chromatography of plasma. Five milliliters of plasma was applied to a 2.2 × 24-cm Spehadex $G25_f$ column (V_t:90 ml) previously equilibrated with a 0.01 M phosphate buffer, pH 7.4, containing 1% bovine serum albumin (Sigma, St. Louis, Missouri), 0.05 M EDTA, 0.15 M NaCl, and 0.02% sodium azide. The column was eluted with the above buffer at room temperature (~23°C) under hydrostatic pressure (60 cm), which resulted in a flow rate of 0.75 ml/min. Fractions were collected in 2.8-ml aliquots and subsequently stored at −20°C until assay. The column was calibrated with Blue Dextran (2,000,000 mol. wt.), ^{125}I glucagon, and synthetic cyclic somatostatin (Bachem, Marena del Rey, California). Chromatography was also performed on 2 × 10-cm Sephadex $G25_f$ column under the same conditions with and without prior incubation of plasma in 8 M urea-1 M KCl at room temperature for 2 h. Fractions (1.2 ml) were collected at a flow rate of 1.5 ml/min and subsequently assayed using antiserum 486.

In additional studies, plasma (2 ml) was subjected to gel filtration on a Sephadex G200 column (0.9 × 110 cm), which had been equilibrated with a 0.01 M phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.05 M EDTA, and 0.02% sodium azide. The column was calibrated with markers of known molecular weight (Pharmacia, Piscataway, New Jersey) and was eluted with the above buffer at room temperature under hydrostatic pressure (20 cm), which resulted in a flow rate of 8 ml/h. Fractions were collected in 1.6-ml aliquots and stored at −20°C until assay. Subsequently, eluates from the above column containing peak I SLI were pooled, concentrated by lyophilization, and reconstituted in 2 ml 0.2 N HCl (pH 2.5), and an aliquot was rechromatographed as above at pH 2.5. Eluates were assayed with antiserum 486.

Radioimmunoassays. Unextracted plasma or aliquots of eluates of chromatographed plasma were subjected to radioimmunoassay using different antisera. The assay employing antiserum 486b has been previously described in detail.[15] The same assay conditions (^{125}I tyrosine 11-somatostatin) were used in studies employing antiserum kindly provided by P. Brazeau (Barbar)[16] and J. Ensinck (AS-10).[17] In studies using antiserum kindly provided by A. Arimura (R-101)[3] and M. Berelowitz (S_16),[2] the same assay conditions were also employed except that ^{125}I N-tyrosine-somatostatin was used as tracer. The antisera were used in final dilutions of 1:15,000(#486), 1:120,000 (Barbar), 1:20,000 (AS-10), 1:10,000 (R-101), and 1:50,000 (S_16). These antisera are sensitive (detection limit 1–4 pg/tube) and are highly specific for somatostatin, although sites of the somatostatin molecule recognized by each differ.[8,15–17]

Recovery studies. Synthetic cyclic somatostatin (100 pg/ml) was added to plasma; one aliquot was frozen for subsequent assay;[15] the other aliquot was frozen, thawed, and immediately subjected to gel filtration as described above. Plasma to which somatostatin had not been added was handled in an identical manner.

Tacer degradation. Unextracted plasma and aliquots of eluates of chromatographed plasma were incubated with ^{125}I tyrosine 11-somatostatin and ^{125}I N-tyrosine-somatostatin under assay conditions. Subsequently, immunoprecipitability of the radiolabeled somatostatins was compared with that observed when the radiolabeled somatostatins were incubated in assay buffer[15] without addition of plasma or chromatographic eluates. Studies of unexpected plasma employed only antiserum 486, whereas studies of chromatographic eluates employed all antisera. Following incubations under assay conditions, the incubate was subjected to gel filtration as described above. All but approximately 5% of added radioactivity eluted as a single peak corre-

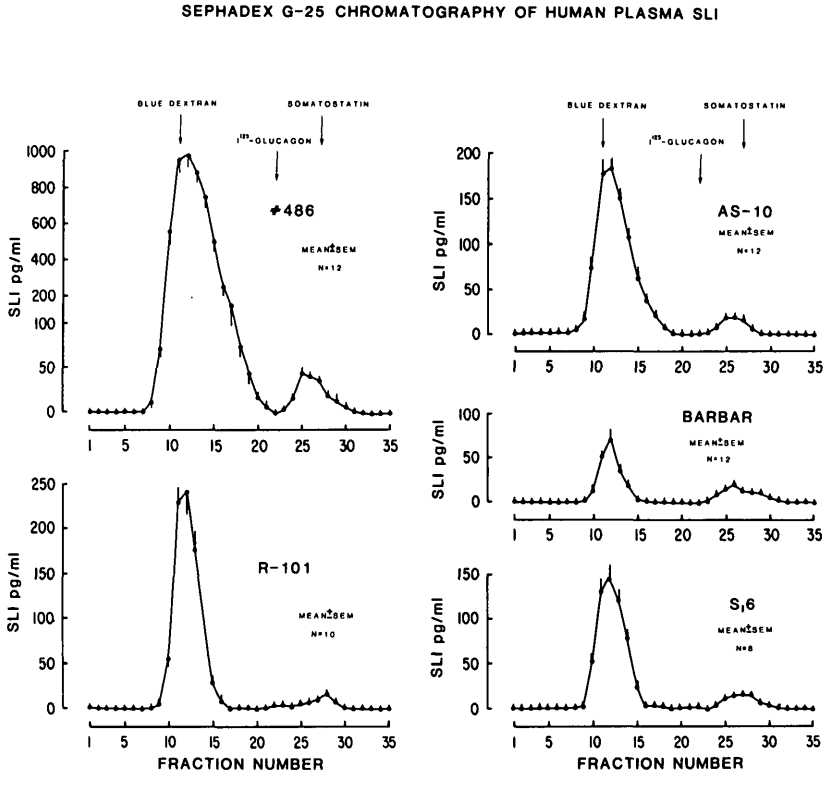


FIGURE 1. Elution profiles of human plasma somatostatin-like immunoreactivity. Plasma (5 ml) was chromatographed on Sephadex $G25_f$ and the resultant eluates were subjected to radioimmunoassay with 5 different antisera (see text for details).

TABLE 1
Relative contributions of peak I and peak II SLI to total SLI in human plasma

Subject	Antiserum									
	R101		S,6		AS10		Barbar		486	
	Peak I	Peak II	Peak I	Peak II	Peak I	Peak II	Peak I	Peak II	Peak I	Peak II
1 f	448	21	260	29	638	20	62	57	3010	130
2 m	580	22	456	36	529	50	152	54	3300	136
3 m	393	32	244	52	520	71	66	79	2860	148
4 m	570	13	291	16	384	95	78	58	3890	140
5 f	603	10	314	27	598	40	147	48	2180	84
6 f	513	18	264	36	406	10	53	36	3000	70
7 m	468	106	230	98	489	40	57	33	3710	60
8 f	667	18	462	32	426	20	171	62	2150	62
9 f	311	21	—	—	471	13	208	62	3310	123
10 f	155	12	—	—	189	12	162	20	4040	65
11 m	—	—	—	—	518	52	101	40	2070	88
12 f	—	—	—	—	455	26	87	72	1900	110
Mean ± SEM	471 ± 48	39 ± 14	315 ± 33	40 ± 8	469 ± 33	37 ± 8	112 ± 15	52 ± 5	2952 ± 213	101 ± 10
Total SLI (Mean ± SEM)	498 ± 49		343 ± 36		506 ± 35		164 ± 16		3052 ± 214	

sponding to intact tracer. The peak was concentrated by lyophilization and reconstituted in assay buffer (3 ml); an aliquot was incubated at 4°C for 24 h in three antisera dilutions, and the amount bound was estimated from the radioactivity precipitated after addition of Dextran-coated charcoal.[15]

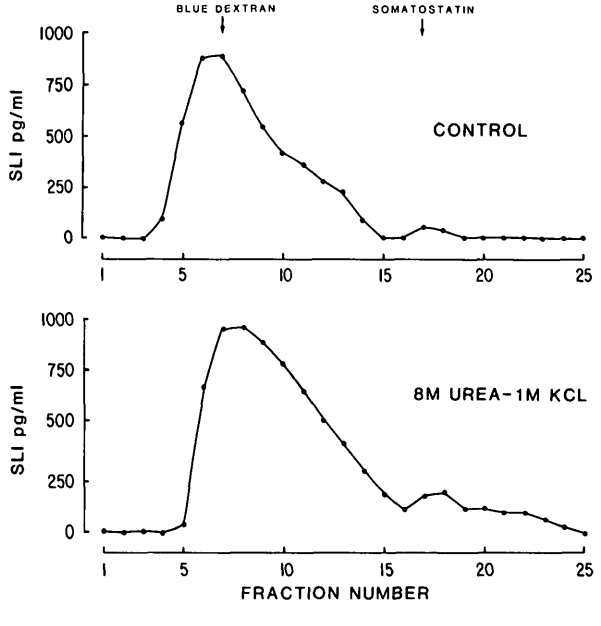
Plasma SLI following infusion of synthetic somatostatin. Blood was collected before and at 60 min during infusion of synthetic cyclic somatostatin (250 μg/h) kindly supplied by R. Guillemin and J. Rivier (Salk Institute, San Diego, California) in an overnight-fasted normal subject as described above; the blood was immediately centrifuged, and the resultant plasma was then subjected to get filtration (Sephadex $G25_f$, see above); eluates were assayed with antisera 486, AS10, and A101.

RESULTS

Gel filtration of human plasma SLI (Figures 1–3, Table 1). The elution profiles of plasma SLI following gel filtration of plasma on Sephadex $G25_f$ are given in Figure 1. With all antisera used, two peaks of SLI were found. One peak (peak I) eluted in the void volume (mol. wt. greater than 5000 daltons); the other peak (peak II) coeluted with synthetic cyclic somatostatin. Chromatography of plasma washed with charcoal removed peak II but did not alter peak I (data not shown). The majority of plasma SLI was due to the void volume material (peak I). The amounts of SLI detected with the various antisera from plasma of overnight-fasted normal subjects are given in Table 1. Mean total plasma SLI (peak I plus peak II) ranged from 164 ± 16 pg/ml (antiserum Barbar) to 3052 ± 214 pg/ml (antiserum 486), depending on the antiserum used. This wide range was due primarily to differences in peak I SLI, which ranged from 112 ± 15 pg/ml (antiserum Barbar) to 2952 ± 213 pg/ml (antiserum 486). Mean values for peak II SLI ranged from 37 ± 8 pg/ml (antiserum AS10) to 101 ± 10 pg/ml (antiserum 486).

The elution pattern of SLI in plasma was not altered when plasma was incubated in 8 M urea-1 M KC1 and subsequently chromatographed (Figure 2). Gel filtration of plasma

FIGURE 2. Elution profiles of human plasma somatostatin-like immunoreactivity without (control) and with 8 M urea-1 M KCl.



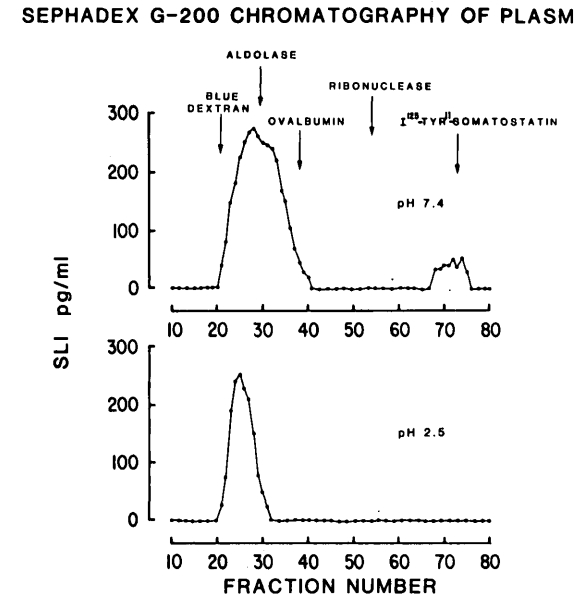


FIGURE 3. Elution profile of human plasma somatostatin-like immunoreactivity following chromatography on Sephadex G200 at pH 7.4 (top); rechromatography of peak I at pH 2.5 (bottom).

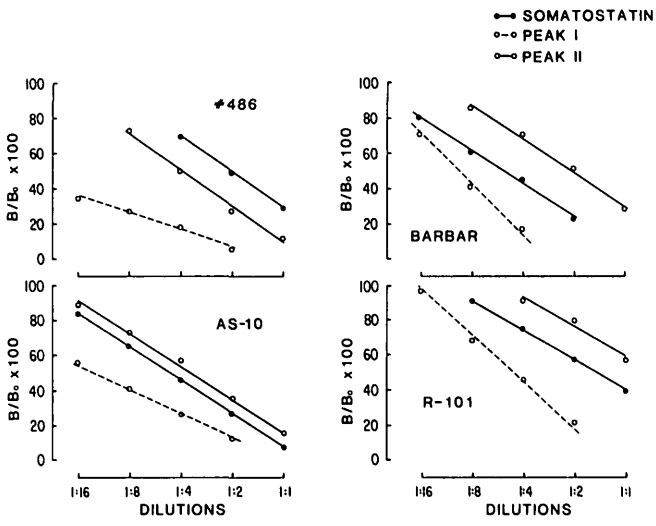
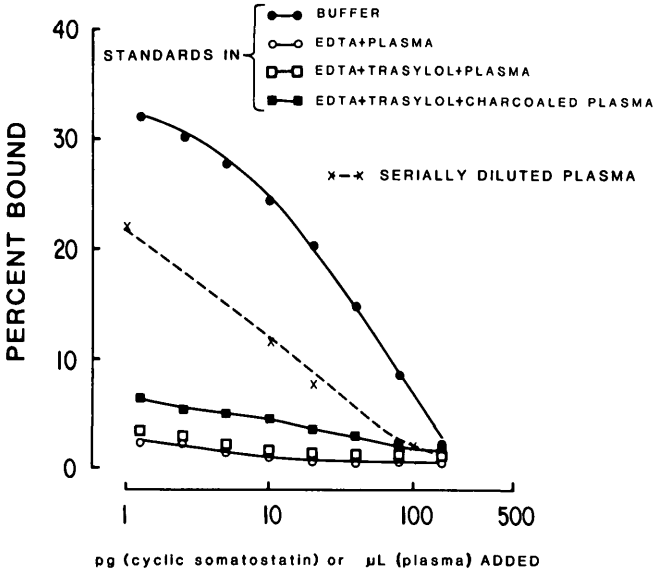


FIGURE 5. Effect of serial dilutions of peak I and peak II plasma somatostatin-like immunoreactivity and of synthetic cyclic somatostatin on binding to four different antisera.

on Sephadex G200 at pH 7.4 also resulted in two peaks of SLI (Figure 3): one peak (peak I) had an apparent molecular weight greater than 150,000 daltons; peak II SLI coeluted with ^{125}I somatostatin; rechromatography of peak I at pH 2.5 resulted in a single peak of SLI that eluted in the same volume as did peak I of plasma SLI chromatographed at pH 7.4

Effect of plasma and peak I and peak II in SLI assay (Figures 4 and 5). Unextracted plasma markedly decreased binding in the 486 antiserum assay; this inhibition was not markedly affected by addition of EDTA, EDTA + trasylol, or by prior treatment of plasma with charcoal (Figure 4); furthermore, SLI in unextracted plasma did not dilute in parallel with synthetic cyclic somatostatin. Peak II SLI, but not peak I SLI, diluted in parallel with synthetic cyclic somatostatin in all four (486, AS10, R-101, Barbar) antisera systems tested (Figure 5).

FIGURE 4. Effect of human plasma on standard curve of somatostatin radioimmunoassay.



Degradation of ^{125}I-labeled somatostatin by unextracted plasma and peak I and peak II SLI (Figure 6). Immunoprecipitability of ^{125}I tyrosine 11-somatostatin and ^{125}I N-tyrosine-somatostatin decreased, respectively, to 40% and 3% after incubation with unextracted plasma for 24 h at 4°C (antiserum 486 1:100). This degradation was not affected by addition of trasylol (1000 KIU/ml) or benzamidine (0.05 M), data not shown. Both ^{125}I-labeled somatostatins were 100% immunoprecipitable when incubated with buffer instead of plasma under the same conditions. Immunoprecipitability of both ^{125}I-labeled somatostatins was also decreased after incubation with peak I in all antisera systems examined (Figure 6). Incubation with peak II decreased immunoreactivity of ^{125}I N-tyrosine somatostatin slightly but not that of ^{125}I tyrosine 11-somatostatin, regardless of the antiserum employed. Addition of trasylol (1000 KIU/ml) did not prevent degradation.

Recovery of somatostatin from plasma (Figure 7). Recovery of synthetic cyclic somatostatin from unchromatographed plasma averaged less than 10%; recovery of synthetic somatostatin added to plasma which was immediately subjected to gel filtration averaged 95 ± 3% (N = 6, range 86–106%). As shown in Figure 7, all of the added synthetic cyclic somatostatin coeluted with peak II SLI.

Effect of infusion of somatostatin on plasma SLI (Figure 8). Infusion of synthetic cyclic somatostatin did not alter peak I SLI with any of the antisera used. Peak II SLI increased from basal values of 12, 12, and 65 pg/ml to 2.06, 2.09, and 2.25 ng/ml at 60 min with antiserum AS10, R-101, and 486, respectively.

DISCUSSION

The present studies confirm previous observations that unextracted human plasma degrades somatostatin (both native hormone and radioiodinated analogues)[1–3] and that it contains substances other than the hormone which interfere or compete with binding of antibody and trace in the soma-

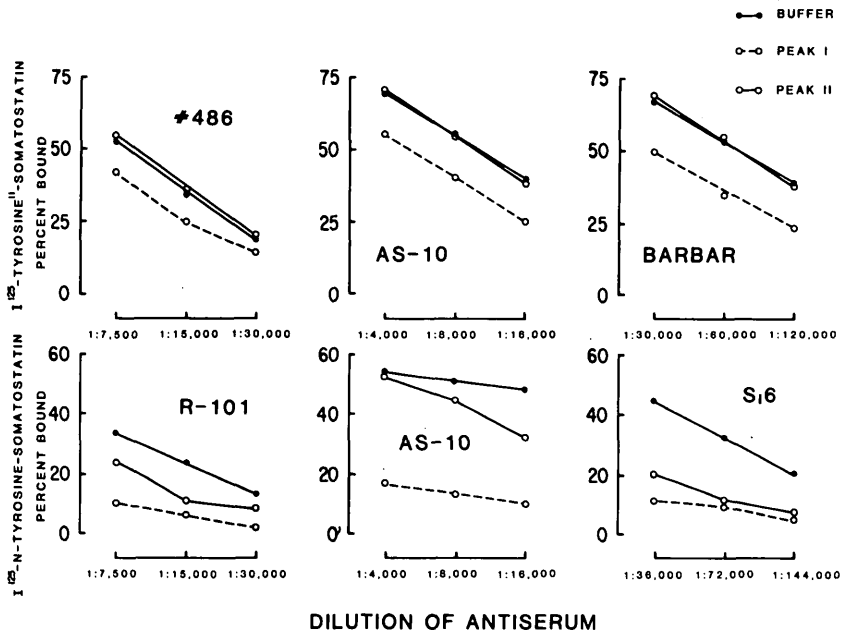
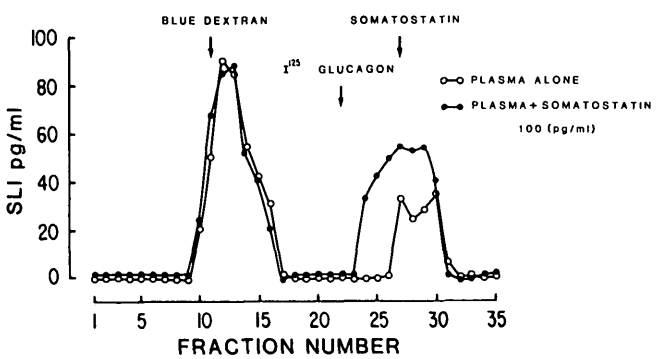


FIGURE 6. Effects of peak I and peak II plasma somatostatin-like immunoreactivity on immunoprecipitability of ^{125}I N-tyrosine somatostatin and ^{125}I tyrosine-11 somatostatin by different antisera.

tostatin radioimmunoassay.[4–9] Thus, in the present studies, recovery of synthetic cyclic somatostatin added to human plasma generally averaged less than 10%; immunoprecipitability of both ^{125}I N-tyrosine somatostatin and ^{125}I tyrosine 11-somatostatin was markedly decreased by human plasma under assay conditions; human plasma somatostatin-like immunoreactivity did not dilute in parallel with synthetic cyclic somatostatin standards in the radioimmunoassay; and, finally, chromatography of human plasma and subsequent assay of eluates with five different antisera demonstrated the presence of two peaks of somatostatin-like immunoreactivity, one eluting with the void volume (mol. wt. > 150,000 daltons) and another coeluting with synthetic cyclic somatostatin in the 1600-dalton region. These findings indicate that unprocessed human plasma cannot be used for reliable determination of circulating somatostatin concentrations by radioimmunoassay.

Extraction of human plasma before assay[6,10] and performance of radioimmunoassay under acid conditions[18] have been used to circumvent degradation and nonspecific interference encountered with unextracted plasma. However, neither of these procedures eliminates the problem of immunoheterogeneity of plasma somatostatin-like immunoreactivity.[9,11–14] In the present studies, synthetic cyclic somatostatin added to human plasma could be quantitatively recovered when plasma was immediately subjected to chromatography. Moreover, somatostatin-like immunoreactivity in plasma corresponding to 1600-dalton material could be separated from other immunoreactivity present in plasma by chromatography and then subjected to radioimmunoassay with negligible tracer degradation. These results indicate that Sephadex chromatography of human plasma before assay may provide a method for avoiding the problems encountered with use of unprocessed human plasma and for permitting reliable determination of circulating somatostatin concentrations in man.

FIGURE 7. Sephadex G25$_f$ elution profile of human plasma somatostatin-like immunoreactivity with (●--●) and without (○--○) addition of synthetic cyclic somatostatin (100 pg/ml).



In the present studies, somatostatin-like immunoreactivity in plasma from postabsorptive normal human volunteers appeared to be predominantly due to immunoreactive material eluting in the void volume after Sephadex G200 chromatography. This material had an apparent molecular weight in excess of 150,000 daltons and was not dissociable by 8 M urea-1 M KCl or by chromatography under acidic conditions (pH 2.5). Values for the amount of this immunoreactivity present in plasma ranged from approximately 100 pg/ml with Barbar antiserum to almost 3000 pg/ml with antiserum 486. However, since this immunoreactivity did not dilute in parallel to standards in any of the five antibody assays studied and since it degraded ^{125}I N-tyrosine somatostatin and ^{125}I tyrosine 11-somatostatin traces, reliable quantitation of the amount of this immunoreactivity present in human plasma is not possible. The nature and physiologic significance of this material remains to be determined. It is likely that the apparent immunoreactivity can be accounted for, at least in part, by the presence of degradative enzymes[1] and a binding protein.[4,5]

In the present studies, human plasma was found to contain somatostatin-like immunoreactivity that eluted on Sephadex chromatography in the 1600-dalton region and diluted in parallel to synthetic cyclic somatostatin standards with all antisera examined. Although this material was not subjected to bioassay, as exogenously infused synthetic cy-

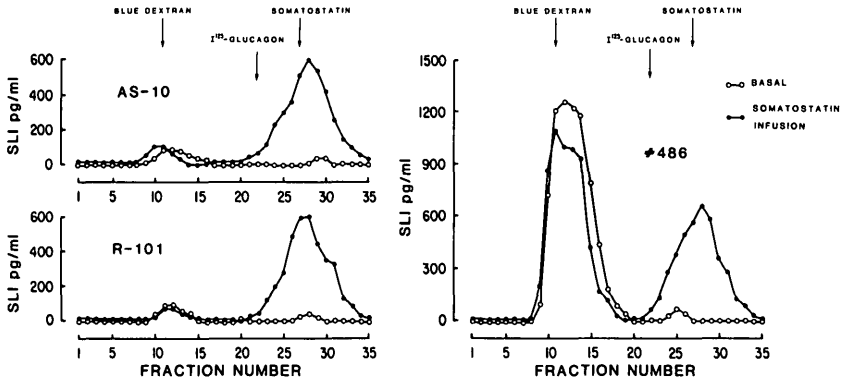


FIGURE 8. Sephadex G25$_f$ elution profile of plasma somatostatin-like immunoreactivity before (O--O) and during (●--●) infusion of synthetic cyclic somatostatin (250 μg/h) in a normal human subject.

clic somatostatin was recovered in the same chromatographic position, it seems likely that most of this material, if not all, could be due to endogenously secreted somatostatin. The concentrations of this somatostatin-like immunoreactivity present in plasma of postabsorptive normal human volunteers ranged from approximately 40 pg/ml with antisera R101, S_16, and AS10 to 100 pg/ml with antiserum 486.

The reason for these differences remains to be determined. It has been suggested that, due to peptidases present in plasma, circulating somatostatin may be in the form of des ala- or des ala, gly-somatostatin.[19,20] Thus, a possible explanation for the differences in peak II immunoreactivity is that antiserum 486 may recognize these peptides or other intermediates that comigrated with somatostatin on Sephadex chromatography to a greater extent than the other antisera. Although antiserum R101,[21] Barbar,[16] S_16,[22] and 486[15] all have been reported to bind N-terminally modified somatostatin less well than somatostatin itself, there has been no systematic assessment of the relative abilities of these antisera to recognize des ala- or des ala, gly-somatostatin. Since both des ala- and des ala, gly-somatostatin are approximately as biologically active as somatostatin itself,[23,24] failure to detect these compounds as somatostatin-like immunoreactivity in plasma could lead to an underestimation of circulating somatostatin biologic activity.

Recently a 28-amino acid N-terminally extended somatostatin has been isolated from gut,[25] hypothalamus,[26,27] and pancreas.[28] This peptide has been reported to be more potent on a molar basis than somatostatin itself in inhibiting growth hormone, insulin, and pancreatic exocrine secretion,[26-30] suggesting that it may not merely be a biosynthetic precursor for somatostatin in the classic sense. However, its biologic role remains to be determined. In none of the assay systems employed in the present study was this larger peptide detected in plasma. Since antiserum 486 and Barbar recognize both the 28- and 14-amino acid peptide equally, it is unlikely that its presence was missed due to differences in antibody specificity. These observations are consistent with several possibilities: the 28-amino acid peptide may not be secreted; it may be secreted in amounts below the detection limit of our assay, or upon secretion, it may be rapidly converted to the 14-amino acid peptide or to a degradative product that is not immunoreactive. The possibility should also be considered that it may circulate in bound form and be part of the somatostatin-like immunoreactive material eluting in void volume chromatographic fractions.

Regardless of the absolute differences in plasma somatostatin concentrations detectable with different antisera, the results of the present study provide additional evidence for a potential hormonal function of somatostatin. Such a role, in addition to that of a neurotransmitter, neurohormone, and local regulatory agent, has been previously postulated.[31,32] In the present studies, mean concentrations of circulating somatostatin found with five different antisera ranged between 40 and 100 pg/ml ($3-6 \times 10^{-11}$ M). Since these were determined under nondissociating conditions, it is likely that they represent free peptide. Concentrations of somatostatin of this order of magnitude are capable of affecting pituitary, gastrointestinal, and pancreatic endocrine function.[33-35] Moreover, in the dog, systemic administration of somatostatin antiserum has been shown to alter gastrointestinal function.[32,36]

In summary, the present study indicates that unprocessed human plasma can degrade native and tyrosylated somatostatin analogues under assay conditions and contains more than one species of immunoreactive somatostatin-like material, only a minor part of which can be accounted for by a 1600-dalton molecule. Sephadex chromatography of plasma before assay, however, circumvents these problems and appears to offer a method for reliable determination of circulating somatostatin. With this technique, circulating concentrations of somatostatin in postabsorptive normal human volunteers were found to be in the range previously reported to exert biologic actions on reputed target tissues, suggesting a possible hormone function for the peptide.

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