Metabolism of recombinant progastrin in sheep

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Paterson, Adrienne C., Graham S. Baldwin, and Arthur Shulkes. Metabolism of recombinant progastrin in sheep. Am J Physiol Endocrinol Metab 283: E449–E456, 2002.—Precursor forms of peptide hormones may be biologically active with effects distinct from the mature end product. Nonamidated progastrin-derived peptides stimulate growth of colonic epithelium and are elevated in the circulation of patients with colorectal carcinomas, whereas the amidated end product is the major regulator of gastric acidity. Using region-specific radioimmunoassays, we here compared the in vitro and in vivo metabolism of recombinant human progastrin-(6–80) and two other nonamidated gastrins, gastrin-17-Gly and Tyr70-progastrin-(71–80). Although progastrin-(6–80) was very stable in vitro, both progastrin-(6–80) and gastrin-17-Gly were degraded in vivo. The in vivo data were best fitted by a double-exponential decay curve, and the half-lives for progastrin-(6–80) (t1/2α = 5.1 ± 1.1, t1/2β = 42 ± 11 min) were significantly (P < 0.05) longer than for gastrin-17-Gly (t1/2α = 2.2 ± 0.6, t1/2β = 13 ± 1 min). Tyr70-progastrin-(71–80) was degraded more rapidly. Comparison with amidated gastrins suggests that peptide length, rather than sequence, is the critical determinant of clearance. Progastrin has the clearance characteristics to be considered a circulating hormone.

PEPTIDE HORMONES ARE PROCESSED from large precursors through a series of intermediates to a final end product that is often amidated (11). Gastrin is synthesized as a large precursor of 101 amino acids, which, after cleavage of the signal peptide, yields progastrin (80 amino acids). Subsequent processing results in the generation of glycine-extended gastrins (gastrin-34-Gly and gastrin-17-Gly), with the final step being amidation to amidated gastrins (gastrin-34 amide and gastrin-17 amide) (Fig. 1) (9, 36). There is increasing evidence that the precursor forms and the end product have distinct biological activities mediated by different receptors (3, 31). Gastrin amide is the major hormonal regulator of gastric acid secretion (13, 36) and acts as a mitogen for normal gastric epithelium and some gastric cancer cell lines in vitro and in vivo (9, 17, 20). The cholecystokinin (CCK)-B/gastrin receptor, which mediates the acid-stimulatory and proliferative effects of gastrin amide is a member of a family of receptors with seven transmembrane segments (21, 39). In contrast, the precursor forms and intermediates such as gastrin-17-Gly and progastrin have little direct effect on gastric acidity (8, 26) but stimulate the proliferation of colorectal carcinomas (both in vitro and in vivo) and normal colon (1, 3, 20, 33). The receptors mediating the effects of gastrin-17-Gly and progastrin have not been defined.

Nonamidated forms of gastrin are present in increased concentrations in the circulation of patients with colorectal carcinoma relative to normal controls (6, 32). Progastrin and gastrin-Gly have also been detected in colorectal tumors (6, 19, 23, 34), but it is still not established whether nonamidated gastrins function as autocrine regulators of tumor development or whether there is also increased secretion from the normal antral source of gastrin (3). In addition, the processing of progastrin is known to be altered in situations of increased gastrin secretion, such as in patients with gastrinomas and in patients or animals with low gastric acidity, often resulting in higher proportions of intermediates (4, 18, 31, 35).

In light of these observations, the determination of the pharmacokinetic properties and biological role of gastrin precursors is of some importance. Studies on the kinetics and biological activity of gastrin-17-Gly have been possible because it can be chemically synthesized (7, 26). However, experiments with progastrin have been very limited, because chemical synthesis of the progastrin polypeptide is not realistic; so the only studies with progastrin have been in transgenic mice overexpressing progastrin (38). It is likely that gastrin-17-Gly and progastrin will have distinct biological activities mediated by separate receptors.

We have developed a method for the purification of progastrin after expression in Escherichia coli (2) to determine its biological activity and to characterize the receptors involved. Here, we present data on the stability of recombinant progastrin in plasma and its pharmacokinetic characteristics in the conscious animal. Because chain length and amino acid derivitization are determinants of peptide metabolism, we have also examined gastrin-17-Gly and a COOH-terminal fragment of progastrin, Tyr70-progastrin-(71–80).

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**MATERIALS AND METHODS**

**Peptides**

Gastrin-17-Gly and Tyr\(^{70}\)-pregastrin-(71–80) were custom synthesized by Auspep (Melbourne, Australia). Recombinant human progastrin-(6–80) was synthesized as a glutathione-S-transferase fusion protein in *E. coli* strain NM522. Briefly, the fusion protein was isolated from the bacterial lysate with glutathione-agarose and cleaved with thrombin. Pregastrin-(6–80) was then purified by reverse-phase HPLC and characterized by radioimmunoassay, amino acid sequencing, and mass spectrometry (2).

**Subjects and Animals**

Fresh human plasma was obtained from healthy adult volunteers. Nonpregnant Merino-Corriedale ewes or plasma from these animals was used for the animal studies.

**Experimental Design**

**Human plasma: in vitro stability.** Approximately 45 ml of peripheral venous blood were taken from the brachial vein and collected into lithium-heparin tubes on ice. The blood was centrifuged at 1,860 \( \times \) g for 15 min at 4°C and the separated plasma stored at −20°C for a maximum of 1 wk before use. Pregastrin-(6–80) [0.5 ml, or 4 pmol/ml, in buffer A (0.02 mol/l veronal, 0.1% BSA, 0.005% sodium azide, pH 8.2)] was added to 19.5 ml of room temperature plasma from each volunteer (\( n = 2 \)) to give a final theoretical concentration of 100 fmol/ml. The plasma was mixed and immediately incubated at 37 or 4°C. One-milliliter samples were taken at 20 and 5 min before 2 ml of recombinant progastrin-(6–80) (250 nM in 0.1% wt/vol BSA in physiological saline) were injected into the right jugular vein, secured, and kept patent with 1,000 U/ml heparin sodium (sample line). Likewise, the infusion line was prepared by cannulating the right jugular vein downward. The next day, the left cannula was cleared of heparin before blood samples were taken at 20 and 5 min before 2 ml of recombinant progastrin-(6–80) (~250 nM in 0.1% wt/vol BSA in physiological saline) were injected into the right cannula. The peptide was flushed with 10 ml of 50 U/ml heparin sodium. This procedure gave an estimated final concentration of 200 pmol/l in the sheep circulation. Samples were taken from the left cannula at 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 60, and 120 min after injection. The line was kept patent with a solution of heparin (50 U/ml) in saline and cleared before and after each time point. Blood was collected into lithium-heparin tubes and placed on ice before centrifugation (as for human plasma). Plasma was stored at −20°C until assay. This procedure was repeated on separate days with the peptides human Tyr\(^{70}\)-pregastrin-(71–80) or human gastrin-17-Gly to give estimated final concentrations of 500 and 200 pmol/l, respectively, in the sheep circulation. At least 24 h separated each experiment.

**Ovine plasma: in vitro stability.** Approximately 100 ml of peripheral venous blood were taken from the jugular vein and collected into lithium-heparin tubes on ice. The blood was centrifuged, separated, and stored as for the human blood. Pregastrin-(6–80) (1 ml, or 4 pmol/ml, in veronal buffer) was added to 39 ml of room temperature plasma from each sheep (\( n = 3 \)) to give a final concentration of 100 fmol/ml. Similarly, 0.5 ml of stock was added to 9.5 ml of buffer A to give a final concentration of 200 fmol/ml (\( n = 2 \)). The solutions were immediately incubated at 37 or 4°C. One-milliliter samples of plasma were taken at −20, 0, and 30 min and 1, 2, 4, 8, 16, and 24 h and ethanol treated as for human plasma for assay with antiserum 1137. Buffer samples were snap frozen in liquid nitrogen.

**In vivo stability.** Sheep were fasted for 18–24 h before the experiment and weighed. Local anesthetic (2 ml of 1% xylocaine) was administered to the skin surrounding the jugular veins. A polyvinyl cannula (0.76 mm ID, 1.6 mm OD) was passed through a needle inserted upward into the left jugular vein, secured, and kept patent with 1,000 U/ml heparin sodium (sample line). Likewise, the infusion line was prepared by cannulating the right jugular vein downward. The next day, the left cannula was cleared of heparin before blood samples were taken at 20 and 5 min before 2 ml of recombinant progastrin-(6–80) (~250 nM in 0.1% wt/vol BSA in physiological saline) were injected into the right cannula. The peptide was flushed in with 10 ml of 50 U/ml heparin sodium. This procedure gave an estimated final concentration of 200 pmol/l in the sheep circulation. Samples were taken from the left cannula at 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 60, and 120 min after injection. The line was kept patent with a solution of heparin (50 U/ml) in saline and cleared before and after each time point. Blood was collected into lithium-heparin tubes and placed on ice before centrifugation (as for human plasma). Plasma was stored at −20°C until assay. This procedure was repeated on separate days with the peptides human Tyr\(^{70}\)-pregastrin-(71–80) or human gastrin-17-Gly to give estimated final concentrations of 500 and 200 pmol/l, respectively, in the sheep circulation. At least 24 h separated each experiment.
**Laboratory Analysis**

**Chromatography.** In vitro samples. Each time point of human plasma from the superspiked in vitro experiments was applied separately to a Sephadex G50 (superfine) column (10 x 1,200 mm; Pharmacia, Uppsala, Sweden), which was eluted at 4°C with 0.02 M veronal buffer containing 0.05% BSA and 0.005% sodium azide, pH 8.2. The flow rate was 7.4 ± 0.1 ml/h (mean ± SD), and 10-min fractions were collected. Blue dextran (Pharmacia) and Na125I (ICN, Sydney, Australia) were used to determine the void and the total volumes, respectively. The columns were calibrated with progastrin-(6-80), Tyr70-progastrin-(71-80), and 125I-labeled gastrin-17 amide. Column fractions were assayed with antisera directed to the COOH- and NH2-terminal parts of progastrin.

In vivo samples. Plasma (2.5 ml) was taken from each progastrin-(6-80) in vivo experiment at +10 min (n = 4) and pooled. Progastrin and derived peptides were concentrated using a C18 Sep-Pak (Waters Associates, Melbourne, Australia) and then applied to a Sephadex G50 column (as described). The flow rate was 4.6 ± 0.1 ml/h, and 10-min fractions were collected. The void volume, total volume, and column calibrations were performed as described. Column fractions were assayed for COOH- and NH2-terminal progastrin and total gastrin.

**Radioimmunoassay.** The four region-specific antisera (Fig. 1) that were used recognized 1) gastrin-Gly (no. 7270), 2) COOH-terminal progastrin (no. 1137), 3) NH2-terminal progastrin (no. 88235), and 4) all molecular forms of gastrin regardless of the degree of processing (total gastrin; no. 8017).

Gastrin-Gly. In vivo plasma samples were measured with antisera 7270 by use of a previously reported radioimmunoassay (7). This antisera detects human but not ovine glycine-extended gastrin and does not cross-react with amidated gastrin or CCK-Gly (Fig. 1) (14). The specific activity of [125I]gastrin-17-Gly was 3.0 ± 0.4 Ci/mmol (n = 2), the ID50 was 1.8 ± 0.4 (n = 4) fmol/ml, and the intra- and interassay coefficients of variation were 7.3 and 7.2%, respectively.

COOH-terminal progastrin. Antisera 1137, which was raised against the COOH-terminal decapetide of human progastrin (6), has a high specificity for intact progastrin and does not detect COOH-terminal-flanking peptides with fewer than eight residues. Antisera 1137 (diluted to 1:200,000) was used to measure progastrin in Sephadex G50 column fractions and in vitro-spiked buffer samples against a buffer A standard curve. Trypsin digestion of the chromatographic fractions was performed according to previously optimized conditions for temperature, buffer, incubation time, and trypsin concentrations (6). Samples were incubated with equal volumes of trypsin (50 µg/ml; Calbiochem, San Diego, CA) in 0.02 mol/l veronal buffer (containing 0.1% charcoal-stripped human plasma and 0.005% sodium azide, pH 8.2) and incubated at 37°C for 2h. Digestion was terminated by boiling for 2 min and then cooling on ice for 5 min. Samples were stored at –20°C until assay. The samples were then radioimmunoassayed using antisera 8017 (diluted 1:2 x 105) against a gastrin-17 amide standard curve, with [125I]gastrin-17 amide as the label (6).

**Calculations**

The pharmacokinetic analysis of Tyr70-progastrin-(71-80) was performed according to a single-compartment, open model (40). This model is described by the equation

\[ C = C_0 \times \exp(-kt) \]

where \( k \) is the slope of the decay curve, \( C_0 \) is the peptide concentration at time zero, and \( C \) is the plasma concentration at time \( t \). The apparent volume of distribution (\( V_d \)) and metabolic clearance rate (MCR) were calculated according to the following formulas, where dose (D) = picomoles injected/weight of sheep.

\[ V_d = \frac{D}{C_0} \]

\[ MCR = k \times V_d \]

The pharmacokinetic analysis of progastrin-(6-80) and gastrin-17-Gly was performed according to a two-compartment model as described by Wartuk (40) and is similar to that reported for gastrin-52 amide (25). This model is described by the equation

\[ C = A \times \exp(-at) + B \times \exp(-bt) \]

where \( a \) and \( b \) are the slopes of the distribution and elimination curves, respectively, and \( A \) and \( B \) are their concentrations at time 0, and \( C \) is the plasma concentration at time \( t \). The MCR was calculated from the injected dose (D) and the area under the curve (AUC) by the equation

\[ MCR = \frac{D}{AUC} = \frac{D}{[(A/a) + (B/b)]} \]

The total apparent \( V_d \) was calculated according to the equation

\[ V_d = \frac{MCR}{k} \]
\[ V_d = \frac{MCR}{\beta} = \frac{D}{\beta}[(A/\alpha) + (B/\beta)] \]

which best describes the pharmacokinetic system when a bolus injection is used (40). The fraction of elimination (F) during the two phases was calculated according to the formulas \( F_a = (A/\alpha)/\text{AUC} \) and \( F_\beta = (B/\beta)/\text{AUC} \) (25). The data were fitted to the equations for the one- and two-compartment models by nonlinear regression with the program Sigmaplot (Jandel Scientific, San Rafael, CA). All results are expressed as means ± SE. Statistical analysis was performed by one-way repeated measures analysis of variance followed by a Tukey's test for pairwise multiple comparisons. P values < 0.05 were considered significant.

RESULTS

In vitro Pharmacokinetics

Recombinant human progastrin-(6–80) appeared to be very stable in human (Fig. 2A) or ovine (Fig. 2B) plasma, with no significant fall in immunoreactivity measured with the COOH-terminal-directed antiserum 1137 after 24 h at either 4 or 37°C. Similarly, recombinant progastrin was stable in ovine blood, with 87 ± 1% (n = 3) of the initial concentration remaining after incubation at 37°C for 24 h. As expected, there was no measurable loss of immunoreactivity when progastrin was incubated in buffer at either 4 or 37°C for 24 h (data not shown).

To confirm that no significant degradation of progastrin had occurred, the progastrin-spiked plasmas after incubation for 0, 2, or 24 h at 37°C were applied independently to a sizing column. The elution positions of progastrin-derived peptides were then determined with antisera directed against the COOH terminus or NH₂ terminus of progastrin (Fig. 3). In the time 0 sample, all of the immunoreactivity co-eluted with intact progastrin when measured with the COOH-terminal antiserum (Fig. 3A). In addition, a minor peak (termed DP2; 16.9% total peak area), reactive only to the NH₂-terminal antiserum 88235, eluted as a broad peak with an elution fraction (Kav) of 0.40 (Fig. 3B). In the 2-h sample, the profile was unchanged with either the COOH-terminal antiserum (Fig. 3C) or the NH₂-terminal antiserum (Fig. 3D; peak area DP2 18.9% total), but in the 24-h sample, the proportion of the NH₂-terminal-reactive peak increased (Fig. 3F; peak area DP2 27.1% total). A new peak also appeared (termed DP1; Kav 0.31), which was detectable only with antiserum 1137 (Fig. 3E). The peak area of progastrin-(6–80) at 24 h, measured by antiserum 1137, decreased by ~10% compared with the time 0 sample, with a corresponding increase in the COOH-terminal-reactive fragment (DP1). The NH₂-terminal antiserum measured similar changes, with an ~10% fall in intact progastrin and a reciprocal increase in the NH₂-terminal-reactive fragment (DP2). On the basis of the log-linear relationship between the elution positions of progastrin-(6–80), gastrin amide, Tyr₇₀-progastrin-(71–80), and Kav, the molecular masses of the COOH-terminal fragment DP1 (Kav 0.31) and the NH₂-terminal fragment DP2 (Kav 0.40) were calculated to be 5,700 and 4,250 Da, respectively.

In vivo Pharmacokinetics

To determine MCR, apparent \( V_d \), and disappearance half-lives, samples of human progastrin, gastrin-17, Gly, and Tyr₇₀-progastrin-(71–80) were injected on separate occasions into four adult ewes. Figure 4 dis-
plays the circulating concentrations of the three peptides at various times after injection.

**Gastrin-Gly.** The basal gastrin-Gly concentration increased from 8 ± 3 pmol/l to a maximum of 142 ± 23 pmol/l after the injection. A biphasic disappearance in the primary or α-phase was 2.2 ± 0.6 min, and in the secondary or β-phase was 13 ± 1 min. The fraction of elimination in the β-phase was 0.83, indicating that the major part of the elimination occurred during this phase. The MCR and Vd were 7.4 ± 1.1 ml·kg⁻¹·min⁻¹ and 142 ± 35 ml/kg, respectively (Table 1).

**Progastrin-(6–80).** The peak level of progastrin immunoreactivity measured with antiserum 1137 was 174 ± 13 pmol/l after injection of 400 pmol of progastrin-(6–80). As for gastrin-Gly, a biphasic disappearance of the peptide from the circulation was observed (Fig. 5B). The half-life in the α-phase was 5.1 ± 1.1 min and in the β-phase 42 ± 11 min. The major part of the elimination (80%) occurred during the β-phase. The MCR and Vd were 2.4 ± 0.2 ml·kg⁻¹·min⁻¹ and 146 ± 40 ml/kg, respectively (Table 1).

The +10 min plasma samples from the four sheep were pooled and applied to a sizing column. The fractions were measured for total gastrin and NH₂-terminal COOH-terminal progastrin (Fig. 6). As for the in vitro samples, a COOH-terminal-reactive peak was seen at a Kᵥ of 0.30 (DP1) (Fig. 6A) and an NH₂-terminal-reactive peak at a Kᵥ of 0.40 (DP2) (Fig. 6B). These peaks comprised 14 and 27%, respectively, of the total immunoreactivity. On the basis of the specificity of the antisera and the estimated molecular masses and on the assumption that cleavage at dibasic residues could occur, likely candidates for DP1 are progastrin-(35–80), progastrin-(36–80), or progastrin-(37–80) and for DP2 progastrin-(6–46), progastrin-(20–54), or progastrin-(20–53). The COOH terminus of DP2 must be at or before residue 55, as DP2 is not detected by antiserum 8017 after trypsin digestion (Fig. 6C).

**Tyr70-progastrin-(71–80).** A monophasic disappearance of the peptide from the circulation was observed (Fig. 5C), in contrast to progastrin-(6–80) and gastrin-17-Gly, which had biphasic disappearance profiles. The disappearance half-life of this 11-amino acid peptide was 0.9 ± 0.1 min, and the MCR and Vd were 187 ± 39 ml·kg⁻¹·min⁻¹ and 235 ± 38 ml/kg, respectively (Table 1).

Comparison of the pharmacokinetic parameters among the different peptides showed that the rate of elimination of progastrin-(6–80) and gastrin-17-Gly could be described using a 2-compartment model, whereas elimination of Tyr70-progastrin-(71–80) followed a 1-compartment model.

![Image](http://ajpendo.physiology.org/)

**Fig. 4.** Disappearance curves of progastrin-derived peptides. Adult ewes were injected with Tyr70-progastrin-(71–80), progastrin-(6–80), and gastrin-17-Gly on separate occasions. Plasma samples were assayed for COOH-terminal immunoreactivity to the respective peptides with Ab 1137 for Tyr70-progastrin-(71–80) (●) and progastrin-(6–80) (○) and Ab 7270 for gastrin-17-Gly (▼). Values are expressed as means ± SE; n = 4.

**Fig. 5.** Logarithmic transformation and regression fitting of the data presented in Fig. 4. The elimination of gastrin-17-Gly (G-Gly; A), progastrin-(6–80) (B), and Tyr70-progastrin-(71–80) (CTFP; C) from plasma of 4 normal ewes, after a bolus injection on separate occasions of each of the peptides, was followed by radioimmunoassay. Values are expressed as means ± SE. The lines of best fit for the distribution (α, dashed line) and elimination ( β, dotted line) phases of a 2-compartment model (A and B) or for a 1-compartment model (C) were obtained by nonlinear regression of the mean values to the equations presented in MATERIALS AND METHODS. Elimination of progastrin-(6–80) and gastrin-17-Gly could be described using a 2-compartment model, whereas elimination of Tyr70-progastrin-(71–80) followed a 1-compartment model.

<table>
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<th>Peptide</th>
<th>t½α, min</th>
<th>t½β, min</th>
<th>MCR, ml·kg⁻¹·min⁻¹</th>
<th>Vd, ml/kg</th>
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<td>Tyr70-progastrin-(71–80)</td>
<td>0.9 ± 0.1*</td>
<td>187 ± 39*</td>
<td>235 ± 38*</td>
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<tr>
<td>Gastrin-Gly</td>
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<td>13 ± 1*</td>
<td>7.4 ± 1.1*</td>
<td>142 ± 35</td>
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<tr>
<td>Progastrin-(6–80)</td>
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<td>42 ± 11</td>
<td>2.4 ± 0.2</td>
<td>146 ± 40</td>
</tr>
</tbody>
</table>

Values are means ± SE of the values obtained from 4 sheep. t½α, Disappearance half-life; MCR, metabolic clearance rate; Vd, volume of distribution. *Significantly different from progastrin-(6–80).
METABOLISM OF RECOMBINANT PROGASTRIN

The nonamidated forms of a number of gastrointestinal-regulatory peptides, including gastrin, secretin, vasoactive intestinal peptide, and gastrin-releasing peptide, are known to be biologically active (29). Gastrin is of particular interest, because the amidated and nonamidated forms have distinct actions that are mediated by separate receptors. Gastrin amide is a hormonal stimulant of gastric acidity that acts via the gastrin/CCK-B receptor, whereas the nonamidated forms have growth-promoting effects on the large bowel (3, 20). The mode of action (autocrine, paracrine, or endocrine) and the nature of the receptors for the nonamidated forms of gastrin have not been determined (3, 10, 20).

We have determined the in vitro and in vivo pharmacokinetics of recombinant progastrin-(6–80) and compared this with another nonamidated form, glycine-extended gastrin-17, and a derivative of the COOH-terminal-flanking peptide (CTFP) of progastrin, Tyr70-progastrin-(71–80). Until the present study, the largest form of nonamidated gastrin for which pharmacokinetics had been determined was glycine-extended gastrin-17 (7, 27), and no studies on progastrin-derived peptides with an unmodified COOH terminus had been reported. The sheep is a suitable model for determining the metabolism of progastrin and progastrin-derived peptides, as multiple blood sampling is practicable and the clearance and processing of glycine-extended and amidated gastrins are comparable among dog, human, and sheep (7, 26, 27). The control systems regulating gastrin release, such as gastric acidity, gastrin-releasing peptide, somatostatin, and acetylcholine are also similar between man and sheep (22, 41, 42).

The removal of progastrin-(6–80) from the circulation followed biphasic exponential kinetics with primary and secondary half-lives of 5.1 and 42 min, respectively. The long disappearance half-life was not the result of the generation of long-lived metabolites, since sizing chromatography of a 10-min blood sample revealed only one other peak comprising <15% of total immunoreactivity. Similarly, progastrin-(6–80) was stable in vitro, with no measurable loss in immunoreactivity after 24-h incubation in plasma or blood at 37°C. In contrast, the CTFP of progastrin, Tyr70-progastrin-(71–80), was rapidly cleared from the circulation, with a disappearance half-life of 0.9 min. These findings are consistent with the proposal that the disappearance half-life of a class of peptide is related to the number of amino acids it contains (12, 15, 37). Accordingly, glycine-extended gastrin-17 had disappearance half-lives (primary, 2.2 ± 0.6 min; secondary, 13 ± 1 min) intermediate between the two progastrin peptides. Until the present study, gastrin-52 amide was the largest gastrin peptide with known pharmacokinetics (24, 25). The disappearance half-lives of gastrin-52 amide (initial 4.9 ± 0.7, secondary 50 ± 4 min) were similar to those of progastrin-(6–80), suggesting that the determining element is peptide length rather than the COOH-terminal sequence. The sites of elimination for progastrin remain to be determined, but the present in vitro studies indicate that circulating enzymes are not responsible. The data from gastrin-52 amide metabolism in pigs indicate that the kidney and head would be the most likely organs to be involved (24).

Comparison of the pharmacokinetics of intact progastrin and its CTFP is of interest, as both are present in the antrum. In the normal human antrum, up to 15% of progastrin immunoreactivity is intact progastrin, as assessed either by specific antisera or by the total gastrin assay (which quantitates a processing-independent fragment of progastrin) (28, 29). The proportion of nonamidated gastrins, including progastrin, is substantially elevated in hypersecretory antral G cells and in gastrinomas and colorectal cancers (31). By use of the total gastrin assay, this increased ratio of nonamidated gastrins is also seen in the plasma of these patients (4, 6, 18). However, there are no reports on the proportions of intact progastrin vs. the CTFP in the circulation, because the total gastrin assays would not detect the smaller form and current CTFP-specific antisera are not suitable for measurement of the low concentrations present in normal serum. The slower clearance of the intact progastrin in the present study would suggest that this form should predominate.
However, formal studies on the relative secretory rates are required. The $V_d$ and MCR for the CTFP were much higher than for the other two peptides, suggesting that the small peptide is being distributed or sequestered to extravascular sites. A similar finding was reported for gastrin-6 amide, again suggesting that size, rather than the nature of the COOH terminus, was the more important determinant (24).

Gel chromatography of progastrin-(6–80) from both the in vivo and in vitro experiments revealed that the majority of the immunoreactivity eluted with intact progastrin-(6–80). Two degradation products were detected, namely a COOH-terminal peak with a $K_v$ of 0.30 (DP1) and an NH$_2$-terminal peak with a $K_v$ of 0.40 (DP2). The small amounts of DP1 and DP2 precluded complete purification and subsequent determination of amino acid sequence, and the multiple potential processing sites (10, 29, 36) mean that a structure based on predicted processing can be of only limited value. Nevertheless, on the basis of the elution positions, antibody specificities, and the assumption that progastrin is cleaved at dibasic residues, the most probable candidate for DP1 is progastrin-(36–80). In agreement with this proposal is the finding that progastrin-(1–35) and progastrin-(6–35) were found as processing products in human gastrinoma extracts (16, 31). It is not clear to what extent infused gastrins are processed at dibasic residues in the circulation. A small proportion of infused gastrin-52 amide is cleaved at Arg$^{36}$Arg$^{37}$ to produce gastrin-34 amide (25), but gastrin-17 amide is not increased after gastrin-34 amide infusion (37). The nature of the NH$_2$-terminal fragment DP2 remains to be determined, but as the NH$_2$-terminal antiserum requires amino acids 20 and 21 to be immunoreactive, possibilities include progastrin-(6–46), progastrin-(20–54), or progastrin-(20–53). The injected progastrin-(6–80) was not processed into gastrin-Gly or -amide in vivo, since no immunoreactivity was detected when the nontrypsinized plasma samples containing the progastrin were assayed with antiserum directed against the NH$_2$-terminal terminus of gastrin-17 (antiserum 8017, data not shown). This observation was confirmed when the sample was chromatographed and assayed for total gastrin. No peaks additional to intact progastrin were detected.

Progastrin-(6–80) and gastrin-(1–17)-Gly both exhibited biphasic disappearance half-lives. The initial phase ($\alpha$-phase) is thought to represent the immediate removal of peptide from the circulation, and the $\beta$, or elimination, phase represents organ-specific metabolism and removal (40). As discussed by Palnæs Hansen et al. (25), the proportion of the peptide cleared in each phase can be determined by the fraction of elimination, Fα and Fβ. Because the Fβs for progastrin and gastrin-Gly were $\sim0.8$, the $t_{\beta/2}$s for progastrin (42 ± 11 min) and gastrin-Gly (13 ± 1 min) are the relevant disappearance half-lives.

In conclusion, the present study shows that full-length progastrin is more stable in the circulation than glycine-extended gastrin-17. In contrast, the CTFP is rapidly metabolized. These results will form a basis for determining whether progastrin has a physiological role as a circulating hormone. However, the circulating forms of progastrin, the extent of postsecretory processing, the sequence required for biological activity, and the nature of the receptors involved all remain unclear. It will be important to address these questions in light of the reports that progastrin and its intermediates accelerate the development of colorectal cancer (3) and sustain acid secretion stimulated by gastrin amide (5).

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