Metabolism of recombinant progastrin in sheep

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First published April 23, 2002; 10.1152/ajpendo.00042.2002.—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) (t1/2A = 5.1 ± 1.1, t1/2B = 42 ± 11 min) were significantly (P < 0.05) longer than for gastrin-17-Gly (t1/2A = 2.2 ± 0.6, t1/2B = 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.

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Peptides

Gastrin-17-Gly and Tyr70-progastrin-(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. Progastrin-(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 g for 15 min at 4°C and the separated plasma stored at −20°C for a maximum of 1 wk before use. Progastrin-(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. 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 Tyr70-progastrin-(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.

Fig. 1. A schematic illustration of the processing of progastrin into mature forms (amidated and glycine-extended gastrins). The sequence specificities of antisera 88235, 8017, 1137, 7270, and 1296 are shown. Antiserum (Ab) 8017 detects a common processing-independent fragment located at the NH2 terminus of gastrin-17 (hatched box), which is exposed by tryptic cleavage. This antiserum enables quantitation of all molecular forms of gastrin (total gastrin), irrespective of the degree of posttranslational processing. Ab 1296 is used to measure fully processed amidated gastrin (the standard gastrin radioimmunoassay) but was not required for this study.

MATERIALS AND METHODS

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. Progastrin-(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.

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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 g for 15 min at 4°C and the separated plasma stored at −20°C for a maximum of 1 wk before use. Progastrin-(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. 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.

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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 × 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 Na-125I (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 antiserum 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 μCi/μmol (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.** Antiserum 1137, which was raised against the COOH-terminal decapptide of human progastrin (6), has a high specificity for intact progastrin and does not detect COOH-terminal-flanking peptides with fewer than eight residues. Antiserum 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. Tyr70-progastrin-(71–80) (Auspep; Melbourne, Australia) was used for the standard curve and 125I-Tyr70-progastrin-(71–80), which was iodinated using a modified chloramine-T method, for the tracer. The specific activity of the labeled peptide was 3.0 ± 0.7 μCi/μmol (n = 2), the ID50 was 2.7 ± 0.5 (n = 4) fmol/ml, and the intra- and interassay coefficients of variation were 3.4 and 8.4%, respectively.

**NH2-terminal progastrin.** Antiserum 88235, which was raised against human progastrin-(20–33), is specific for the sequence 20–33 of human progastrin. The antiserum detects the pentadecapeptide 20–33 and progastrin-(20–72) amide equally well. Progastrin fragments extended NH2 terminally to 20–33 have ~50% cross reactivity, whereas cross reactivity to progastrin fragments without amino acid 20 is 10% and without residues 20 and 21 is 0.1% (30). Antiserum 88235 (diluted to 1:500,000) was used to measure progastrin in Sephadex G50 column fractions against a buffer A standard curve constructed with Tyr70-progastrin-(20–34) (kindly supplied by Jens Rehfeld) as standard and [125I]Tyr70-progastrin-(20–34) as tracer.

**Total gastrin.** Antiserum 8017 detects the NH2-terminal portion of gastrin-17, which is exposed after tryptic digestion (Fig. 1). Hence, all unprocessed, partially processed, and mature forms of gastrin (total gastrin) are measured with this procedure. Tryptic digestion of the chromatographic fractions was performed using 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 2 h. 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 antiserum 8017 (diluted 1:2 × 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

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

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

\[ C = A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t) \]

where \( \alpha \) and \( \beta \) are the slopes of the distribution and elimination curves, respectively, \( A \) 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

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

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

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

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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. 3E; 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 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 Vd, and disappearance half-lives, samples of human progastrin, gastrin-17, gastrin amide, 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 of the peptide from the circulation was observed for each of the four sheep (Fig. 5A). The disappearance half-life in the primary or α-phase was 2.2 ± 0.6 min, and in the secondary or β-phase 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- and 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 antiseras 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

#### Table 1. Pharmacokinetic properties of Tyr70-progastrin-(71–80), gastrin-17-Gly, and progastrin-(6–80)

<table>
<thead>
<tr>
<th></th>
<th>tα, min</th>
<th>tβ, min</th>
<th>MCR, ml·kg⁻¹·min⁻¹</th>
<th>Vd, ml/kg</th>
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</thead>
<tbody>
<tr>
<td>Tyr70-progastrin-(71–80)</td>
<td>0.9 ± 0.1*</td>
<td>187 ± 39*</td>
<td>235 ± 38*</td>
<td></td>
</tr>
<tr>
<td>Gastrin-Gly</td>
<td>2.2 ± 0.6*</td>
<td>13 ± 1*</td>
<td>7.4 ± 1.1*</td>
<td>142 ± 35</td>
</tr>
<tr>
<td>Progastrin-(6–80)</td>
<td>5.1 ± 1.1</td>
<td>42 ± 11</td>
<td>2.4 ± 0.2</td>
<td>146 ± 40</td>
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Values are means ± SE of the values obtained from 4 sheep. tα, Disappearance half-life; MCR, metabolic clearance rate; Vd, volume of distribution. *Significantly different from progastrin-(6–80).
Assay of progastrin (Ab 1137; A)

Fractions were assayed with antisera directed to the COOH terminal after a bolus injection of human recombinant progastrin-(6–80). The metabolism of recombinant progastrin-(6–80) 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.

DISCUSSION

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).

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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 for recognition, it is possible that DP2 is a fragment released from the COOH terminus and that the NH$_2$-terminal antiserum recognizes amino acids 20 and 21.

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 uncertain. 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 amid (5).

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