Excretion of progastrin products in human urine

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Palnaes Hansen, C., J. P. Goetze, F. Stadil, and J. F. Rehfeld. Excretion of progastrin products in human urine. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G985–G992, 1999.—The renal handling of carboxyamidated gastrins, NH2-terminal progastrin fragments, and glycine-extended gastrins was examined in healthy volunteers. The respective urinary clearances after a meal amounted to 0.09 ± 0.02% (P < 0.05), and 0.04 ± 0.01% (P < 0.01) of the glomerular filtration rate. During intravenous infusion of carboxyamidated gastrin-17, progastrin fragment-(1—35), and glycine-extended gastrin-17, the respective urinary clearances amounted to 0.08 ± 0.02, 0.46 ± 0.08, and 0.02 ± 0.01%, respectively, of the glomerular filtration rate. The metabolic clearance rate of the three peptides was 24.4 ± 1.3, 6.0 ± 0.4, and 8.6 ± 0.7 ml·kg−1·min−1. A maximum rate for tubular transport or degradation of the peptides could not be determined, nor was a renal plasma threshold recorded. Plasma concentrations and urinary excretion rates correlated for gastrin-17 and progastrin fragment-(1—35) (r = 0.94 and 0.97, P < 0.001), whereas the excretion of glycine-extended gastrin diminished with increasing plasma concentrations. We conclude that renal excretion of progastrin products is negligible compared with renal metabolism and that renal handling of the peptides depends on their molecular structure. Hence, the kidneys exhibited a higher excretion of NH2-terminal progastrin fragments than of carboxyamidated and especially glycine-extended gastrins.

THE KIDNEYS ARE active in the metabolism of gastrin. Canine and porcine renal extraction of carboxyamidated gastrin-17 varies from 9 to 40%, but <0.4% of the filtered gastrin-17 is present in the urine (4, 5, 22, 23, 31). This implies either complete absorption of filtered gastrin-17 in the renal tubules or excretion of nondetectable degradation fragments in the urine. In humans, the kidneys also seem to be important regulators of plasma gastrin levels as inferred from the elevated concentrations of gastrin in patients with renal failure (19, 32). Like in other mammals, the concentration of gastrin peptides in human urine is usually low; however, analysis of postprandial urine has revealed significant differences between the excretion of COOH- and NH2-terminal fragments of gastrin (11, 12). Until now, urinary excretion of gastrin in humans has only been investigated in postprandial urine or in urine from patients with hypergastrinemia (8, 11, 12, 27). Intravenous administration of gastrin enables a more detailed information about renal handling of different gastrin peptides.

To find out whether urinary excretion of gastrin is related to either the molecular form or to bioactivity of the peptides, we studied endogenous gastrins in postprandial urine from normal subjects as well as urinary excretion of gastrin during intravenous administration of three different products of progastrin. The first product was carboxyamidated gastrin-17 (gastrin-17), which is the bioactive main product. The second was glycine-extended gastrin-17 (gastrin-17-Gly), which is the immediate precursor of gastrin-17 (15). Glycine-extended gastrins are cosecreted in small amounts along with carboxyamidated gastrin and are not bioactive with regard to acid secretion (13, 21), although it has been suggested that glycine-extended gastrins may stimulate cellular growth (28). The third product was the NH2-terminal fragment-(1—35) of progastrin [progastrin-(1—35)], which circulates in plasma with other NH2-terminal fragments of progastrin (26). So far, no biological function has been connected with these fragments (Fig. 1).

MATERIALS AND METHODS

Peptides

Human carboxyamidated nonsulfated gastrin-17 was purchased from Sigma (St. Louis, MO). Glycine-extended human nonsulfated gastrin-17 and human progastrin-(1—35) were custom synthesized by Cambridge Research Biochemicals (Zeneca, Alderley Park, Cheshire, UK). The content and purity of the peptides were controlled by amino acid analysis (LKB amino acid analyzer 4000 with fluorescence detection, LKB Biochrom, Cambridge, UK) and reverse-phase HPLC (Hewlett Packard 1084B, Palo Alto, CA).

After sterile filtration, the peptides were diluted in isotonic saline containing 1 g/l human serum albumin. The syringes were weighed before and after the infusions, and the remaining peptide from the infusion lines was stored at −20°C until radioimmunoassay.

Subjects

The studies were carried out in healthy subjects without a history of medical or surgical illness. Informed consent was obtained, and the studies were approved by the Ethics Committee for Medical Research in Copenhagen in accordance with the Helsinki II declaration.

Experiments

Recovery of gastrin in urine. Recovery was measured by adding gastrin-17, gastrin-17-Gly, and progastrin-(1—35) separately to urine and assay buffer in different concentrations. Degradation with time was investigated by incubating the peptides in urine for 1, 2, 4, 8, and 24 h. After incubation, all samples were immediately frozen in liquid nitrogen and stored at −20°C until analysis.

Renal excretion of endogenous gastrin. Renal excretion of postprandial gastrin was studied in seven subjects (23–33 yr, body surface 1.81 ± 0.05 m2). After an overnight fast, each

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Renal excretion of gastrin-(1—35) and gastrin-17-Gly. Renal excretion of gastrin-(1—35) and gastrin-17-Gly was studied in eight subjects (23–28 yr, body surface 1.87 ± 0.09 m²). Each peptide was infused simultaneously at three consecutive dose rates, each lasting 90 min [progastrin-(1—35): 15, 30, and 60 pmol·kg⁻¹·h⁻¹; gastrin-17-Gly, 30, 60, and 120 pmol·kg⁻¹·h⁻¹]. Urine and blood samples were drawn as stated above. GFR was measured by a single injection of ⁵¹Cr-EDTA.

Laboratory Analyses

Radioimmunoassay. Three sequence-specific antisera were used to measure the peptides in plasma and urine. Gastrin-17 was measured using antisera 2604, which was raised against the 2–17 fragment of human gastrin-17 and is specific for the bioactive COOH terminus. The antisera binds gastrin-71, -52, -34, and -17 with equimolar potency, and its reactivity with cholecystokinin peptides is <0.5% (30). Synthetic human gastrin-17 was used as a standard, and monoiiodinated ¹²⁵I-progastrin-17 was used as tracer (29).

Gastrin-17-Gly was measured using antisera 7270. This antisera was raised against the 5–17 fragment of gastrin-17-Gly. Gastrin-17-Gly was used as standard and monoiiodinated ¹²⁵I-gastrin-17-Gly as tracer (14). Antiserum 7270 does not recognize amidated gastrins, cholecystokinin, or glycine-extended cholecystokinin.

Progastrin-(1—35) was measured using antisera 88235, which was raised against fragment 20–33 of human progastrin that corresponds to the NH₂ terminus of gastrin-52. The antisera is specific for the sequence 20–25 of progastrin, but peptides extended NH₂-terminally to this sequence are also bound, although with lower affinity. The antisera does not bind gastrin-34, -17, or smaller carboxyamidated gastrins, nor does it react with cholecystokinin (20, 26). Synthetic human gastrin-52 was used as standard, and monoiiodinated ¹²⁵I-progastrin-52 was used as tracer. Because progastrin-(1—35) is extended NH₂-terminally of the epitope 20–25 of progastrin, tryptic and carboxypeptidase B cleavage before measurement were necessary for accurate quantitation (14).

Plasma was incubated with equal volumes of trypsin (2 mg/ml) (Worthington Diagnostic Systems, Freehold, N.J.) and 0.05 M Na₂HPO₄ at pH 7.5 and 20°C for 30 min. The trypsin solution was terminated by boiling for 10 min. After centrifugation, the supernatant was incubated with carboxypeptidase B (5 mg/ml) (Boehringer Mannheim) 100 µl/ml sample at 20°C for 30 min. The reaction was terminated by boiling for 10 min.

Urine analysis. After measurement of urine volume, samples were collected in ice-chilled tubes and stored at −20°C until analysis. The urine was concentrated, and the peptides were extracted using octadecylsilicayl silica columns (Sep-Pak C₁₈ Cartridges, Waters Associates, Milford, MA) (8). The cartridges were prewashed with 10 ml of 75% alcohol at 0.01 M HCl followed by 10 ml distilled water. Volumes of urine up to 20 ml were filtered through the cartridges with a flow rate of 10 ml/min. After being washed with 10 ml distilled water, the peptides were eluted with 40 ml of 75% alcohol in 0.01 M HCl. The eluate was dried under air flow and reconstituted in assay buffer. The concentration of the progastrin products in urine was measured by radioimmunoassay.

Chromatography. Plasma and urine samples of 1 ml were applied to Sephadex G-50 superfine columns (10 × 1,000 mm, Pharmacia, Uppsala, Sweden) and eluted with 0.125 M NH₄HCO₃ (pH 8.2) at room temperature with a flow rate of 3 ml/h. Void volume and total volume were determined by elution of ¹₂⁵I-albumin and ⁵¹NaCl. The columns were calibrated with gastrin-17, gastrin-17-Gly, and progastrin-(1—35) as standards.
Fig. 1. Recovery of gastrin in urine.

Table 1. Recovery of gastrin in urine

<table>
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<tr>
<th>Hours</th>
<th>Gastrin-17, %</th>
<th>Progastrin-(1–35), %</th>
<th>Gastrin-17-Gly, %</th>
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<td>104 ± 4</td>
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<td>98 ± 2</td>
<td>86 ± 3</td>
<td>94 ± 12</td>
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<tr>
<td>24</td>
<td>104 ± 6</td>
<td>99 ± 6</td>
<td>106 ± 14</td>
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Values are means ± SE; n = 6 experiments. Concentrations of peptide at time 0: gastrin-17, 168 ± 20 pM; progastrin-(1–35), 103 ± 4 pM; gastrin-17-Gly, 242 ± 15 pM. Gastrin-Gly, glycine-extended gastrin.

Renal Excretion of Endogenous Gastrin

The basal concentrations of carboxyamidated gastrins, NH₂-terminal progastrin fragments, and glycine-extended gastrin in plasma were 10 ± 2, 12 ± 2, and 6 ± 1 pM, respectively. During the meal, carboxyamidated gastrins and NH₂-terminal progastrin fragments in plasma reached peak concentrations of 25 ± 4 and 16 ± 4 pM (P < 0.01), respectively, whereas the concentration of glycine-extended gastrin remained almost constant (Fig. 3). The average GFR was 98.1 ± 1.7 ml·min⁻¹·1.73 m², and urinary flow was 53.6 ± 0.6 ml/min. Basal excretion rates of carboxyamidated gastrins, NH₂-terminal progastrin fragments, and glycine-extended gastrin were 1.370 ± 0.267, 1.935 ± 0.579, and 0.467 ± 0.300 fmol/min, respectively. The postprandial excretion rate of carboxyamidated gastrins and NH₂-terminal progastrin fragments reached a maximum of 2.416 ± 0.940 fmol/min (0.10 > P > 0.05) and 3.854 ± 1.339 fmol/min (P < 0.05), respectively, whereas the excretion rate of glycine-extended gastrin displayed a gradual decrease to 0.082 ± 0.018 fmol/min at the final sample (P < 0.05). The average urinary clearances of postprandial carboxyamidated gastrins, NH₂-terminal progastrin fragments, and glycine-extended gastrins were (in ml·min⁻¹·1.73 m²) 0.093 ± 0.014, 0.164 ± 0.041 (P < 0.05), and 0.039 ± 0.012 (P < 0.01), respectively, equivalent to 0.09 ± 0.02, 0.17 ± 0.04, and 0.04 ± 0.01%, respectively, of the GFR. The peptide concentrations of gastrin in plasma and urine were too low for gel chromatography.

Renal Excretion of Gastrin-17

Gastrin-17 was infused at three consecutive dose rates of 58 ± 5, 115 ± 11, and 223 ± 12 pmol·kg⁻¹·h⁻¹. The average MCR of the peptide at steady state was 24.4 ± 1.3 ml·min⁻¹·kg⁻¹. Urinary excretion rate of gastrin-17 increased concomitantly with the stepwise rise of peptide in plasma (Fig. 4), with correlation between excretion rate and plasma concentration (r = 0.94, P < 0.001; Fig. 5). A maximum tubular absorption (Tₘ) value was not achieved within the present dose rates. The average GFR and urinary flow values were 98.3 ± 4.3 ml·min⁻¹·1.73 m² and 5.1 ± 0.3 ml/min, respectively, and the average urinary clearance of...
gastrin-17 amounted to $0.075 \pm 0.016$ ml·min$^{-1}$·1.73 m$^{-2}$, equivalent to $0.08 \pm 0.02\%$ of GFR (Table 2).

Gel chromatography of plasma revealed a major peak in the elution position of gastrin-17 (Fig. 6). The concentration of gastrin-17 in urine was too low for gel chromatographic analysis.

Renal Excretion of Progastrin-(1—35) and Gastrin-17-Gly

The peptides were administered simultaneously. The consecutive dose rates of progastrin-(1—35) were 15 ± 1, 29 ± 2, and 62 ± 3 pmol·kg$^{-1}$·h$^{-1}$, and the dose rates...
MCR of progastrin-(1—35) at steady state was 6.0 reached within the present dose rates. The average ml·min\(^{-1}\) and significantly lower than Cl\(_{u}/GFR\) (%) for gastrin-17 and progastrin-(1—35) (\(P<0.001\); Fig. 8). A \(T_{m}\) value was not reached within the present dose rates. The average MCR of progastrin-(1—35) at steady state was 6.0 ± 0.4 ml·min\(^{-1}\)·kg\(^{-1}\), and the average urinary clearance was 0.430 ± 0.057 ml·min\(^{-1}\)·1.73 m\(^{-2}\), equivalent to 0.46 ± 0.08% of GFR (Table 2). This value was significantly higher than Cl\(_{u}/GFR\) (%) for gastrin-17 (\(P<0.01\)).

Unlike gastrin-17 and progastrin-(1—35), there was no rise in urinary excretion of gastrin-17-Gly with increasing dose rates of the peptide, although the plasma concentrations reached high levels during infusion. After the start of the two first dose rates, a minor rise in the urinary excretion rate of gastrin-17-Gly was followed by a gradual fall (\(P<0.05\); Fig. 9). The average MCR at steady state was 8.6 ± 0.7 ml·kg\(^{-1}\)·min\(^{-1}\), and the average urinary clearance was 0.016 ± 0.005 ml·min\(^{-1}\)·1.73 m\(^{-2}\), equivalent to 0.02 ± 0.01% of GFR (Table 2) and significantly lower than Cl\(_{u}/GFR\) (%) for gastrin-17 and progastrin-(1—35) (\(P<0.01\)). The plasma concentration of carboxyamidated gastrins remained constant during infusion of progastrin-(1—35) and gastrin-17-Gly, whereas urinary excretion apart from an initial fall oscillated during the study. Urinary excretions of gastrin-17-Gly and carboxyamidated gastrins were not correlated.

Gel chromatography of plasma revealed major peaks in the elution positions of progastrin-(1—35) and gastrin-17-Gly (Fig. 6). The concentrations of the peptides in urine were too low for gel chromatography.

**DISCUSSION**

The present results showed that urinary excretion of intact progastrin products contributes only little to the metabolic clearance rate of postprandially released and infused gastrin in humans. The low concentration in urine was not due to analytical bias, since urine did not influence immunoreactivity and measurement of the peptides. The excretion of carboxyamidated gastrins and \(NH_{2}\)-terminal progastrin fragments increased postprandially as well as during infusion of gastrin-17 and progastrin-(1—35). However, the ratio of urinary clearance to GFR of carboxyamidated gastrins and \(NH_{2}\)-terminal progastrin fragments differed significantly, with the \(NH_{2}\)-terminal progastrin fragments being twice that of carboxyamidated gastrins during meal stimulation and nearly six times higher for progastrin-(1—35) than for gastrin-17. Urinary clearance of glycinextended gastrins was significantly lower than the clearances of carboxyamidated gastrins and of \(NH_{2}\)-terminal progastrin fragments, and urinary excretion decreased both postprandially and during infusion of gastrin-17-Gly. Conversion of gastrin-17-Gly to the amidated form in the nephron was unlikely, since the urinary excretion of amidated gastrin was independent of the plasma concentration of gastrin-17-Gly. Although the urinary excretion of intact peptides only constituted a small fraction of the filtered amount, the results indicate that urinary clearance of progastrin-derived peptides depends on their molecular structure.

The MCR values of gastrin-17, gastrin-17-Gly, and progastrin-(1—35) were as previously recorded in humans. Because neither renal plasma flow nor arterial plasma concentration of gastrin was measured, renal metabolism of gastrin can only roughly be estimated. With the assumption of a renal plasma flow of 12 ml·kg\(^{-1}\)·min\(^{-1}\) and a fractional extraction of 0.4, total renal clearance of gastrin, which includes metabolism and excretion, would be 5 ml·kg\(^{-1}\)·min\(^{-1}\) or ~20% of the total body clearance. Plasma concentrations used in pharmacokinetic calculations have to represent the concentration at the metabolic inlet. Because the clearance of gastrointestinal peptides also occurs in non-splanchnic tissues such as extremities (23, 24, 30), the calculated MCR will be overrated if venous plasma from an arm vein rather than arterial plasma is used. The same problem complies with calculation of urinary clearance. Therefore, the estimated values of MCR and urinary clearance of gastrin may be somewhat higher than under ideal experimental conditions. Studies in pigs have revealed an identical extraction ratio of gastrin-17 and gastrin-17-Gly in the limbs (23). If
extraction of gastrin also occurs in the human extremities, it is anticipated that the bias in calculating MCR and urinary clearance will be of the same magnitude for the two peptides.

Renal clearance of peptides is accomplished by glomerular filtration with subsequent absorption and degradation in the proximal tubules of the nephron (3, 6, 9, 16). Several membrane-bound peptidases have been isolated from the kidneys (10, 34). Thus filtered progastrin products would be either reabsorbed and metabolized in the tubules or degraded intraluminally by peptidases in the brush border of the tubular cells and then absorbed or excreted as degraded fragments in urine. Investigations of radiolabeled gastrin-17 suggest that at least part of the peptide is absorbed into tubular cells (18). Uptake of gastrin may be initiated by binding to nonspecific membrane sites at the brush border of the tubular cells or to specific receptors. Gastrin/cholecystokinin B receptor expression has been demonstrated in renal tissue (35). However, we have not been able to show receptor binding for either carboxyamidated or glycine-extended gastrin in renal tissue (unpublished data). Consequently, it is still unclear whether absorption of progastrin products is facilitated by specific receptors.

An earlier study found NH$_2$-terminal octa-, nona-, and decapeptide fragments of gastrin-34 in postprandial human urine in concentrations several hundred times that of carboxyamidated gastrin (11). It was suggested that NH$_2$-terminal gastrin fragments were less well absorbed in the tubules than COOH-terminal fragments. In addition, it has been assumed that fragments without biological activity extracted by the kidneys were mainly excreted in urine rather than reabsorbed and metabolized in analogy with the inactive C-peptide of proinsulin (25). Our results confirm that NH$_2$-terminal progastrin fragments including progastrin-(1—35) are excreted in urine in larger amounts than carboxyamidated gastrins, although the difference was less pronounced than reported for small NH$_2$-terminal fragments of gastrin-34. The difference...
in urinary clearance was hardly related to bioactivity regarding acid secretion, since the inactive gastrin-17-Gly displayed the lowest urinary clearance of the three peptides.

Urinary clearance has no meaning as an indicator of renal elimination for substances metabolized by the kidney. However, a comparison of urinary clearance with GFR provides a measure of the fraction excreted relative to the amount filtered. Urinary clearance of gastrin-17, gastrin-17-Gly, and progastrin-(1—35) comprised 0.08, 0.02, and 0.50%, respectively, of GFR, which indicates either a high degree of tubular absorption or urinary excretion of degradation fragments that are not measured by the assays used in this study. The glomerular membrane functions both as a size-selective and as a charge-selective barrier. Negatively charged macromolecules are restricted to a greater extent than neutral or positively charged molecules of the same size because of negative charges in the glomerular membrane (6, 7). For proteins with a molecular mass less than 15 kDa, the charges of the glomerular membrane have no appreciable effect on the glomerular filtration, whereas the forces of diffusion increase with decreasing molecular size. This has been shown with the anionic polypeptide aprotinin (33) and is a result of the Gibbs-Donnan equilibrium between plasma and the almost protein-free ultrafiltrate of Bowman’s space, which tends to restrict the filtration of cations but to enhance filtration of anions over the glomerular membrane. The filtered load of a substance equals GFR multiplied by the plasma concentration and the fraction of the filtered substance in relation to glomerular plasma (GFR × P × f). For small charged molecules, the filtration fraction f depends on binding to plasma proteins as well as the Donnan ratio, r2, which is determined by the net anion concentration from plasma proteins and the salt concentration (22, 33). Under physiological conditions, this ratio is ~1.05. Because the small peptides gastrin-17 (2.1 kDa), gastrin-17-Gly (2.2 kDa), and progastrin-(1—35) (3.9 kDa) carry a net negative charge (z = −6, −7, and −1, respectively) and because they are not bound to plasma proteins, the filtration fraction is equivalent to the Donnan ratio. This is equivalent to 1.05 for progastrin-(1—35) and can be estimated for gastrin-17 and gastrin-17-Gly to be 1.34 and 1.41, respectively. The calculations suggest that the filtration rate of gastrin-17 and gastrin-17-Gly should exceed that of progastrin-(1—35) with 28 and 34%, respectively, provided an identical GFR and plasma concentration in the glomerular capillaries. Thus differences in urinary clearance result from both glomerular as well as tubular handling of the peptides. In spite of a high filtration fraction, gastrin-17-Gly had the lowest urinary clearance of the three peptides. This might be explained in part by the different COOH- and NH2-terminal derivatization of the peptides that may influence their handling in the renal tubules. Gastrin-17 and gastrin-17-Gly are both protected against degradation from aminopeptidases by the NH2-terminal pyroglutamyl residue. Gastrin-17 is also protected against degradation by carboxypeptidases due to the amidation of its COOH-terminal carboxyl group. This makes gastrin-17-Gly more susceptible to COOH-terminal degradation into fragments excreted either in
urine or absorbed in the renal tubules, fragments which we at present are unable to measure. Contrary to earlier observations, we found no evidence of a renal plasma threshold for progastrin products (27). However, a spillage was constantly present, which correlated linearly with the concentration of gastrin-17 and progastrin-(1—35) in plasma. Absorption and degradation of peptides in the nephron are assumed to be controlled by saturable processes. Consequently, below-saturation absorption increases continuously with the filtered load, and after saturation, the excretion parallels the rate of filtration of the substance (16). In spite of supraphysiological plasma concentrations of the three peptides, a Tm level for tubular transport or degradation was not achieved.

We conclude that the urinary clearance of progastrin-derived peptides varies with their molecular structure under the influence of the glomerular filtration and the handling of the peptides in the renal tubules. Moreover, our results show that the capacity of tubular transport and metabolism is high and far from being saturated under normal physiological conditions. Bioactivity seemed to be without influence on the renal handling of the peptides.

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