The metabolism of gastrin-52 and gastrin-6 in pigs

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Palnæs Hansen, C., J. P. Goetze, F. Stadil, and J. F. Rehfeld. The metabolism of gastrin-52 and gastrin-6 in pigs. Am J Physiol Gastrointest Liver Physiol 279: G552–G560, 2000.—The kinetics and metabolism in various organs of three bioactive products of progastrin, the small sulfated and nonsulfated gastrin-6 and the large nonsulfated gastrin-52, were examined during intravenous administration in anesthetized pigs. The kidney, hindlimb, liver, head, and gut eliminated the hexapeptides efficiently, with a fractional extraction ranging from 0.50 to 0.28 (P < 0.001–0.05). No metabolism was recorded in the lungs, and sulfation was without influence on the extraction of gastrin-6. Gastrin-52 was eliminated only in the kidney and the head, with a fractional extraction between 0.23 and 0.11 (P < 0.01–0.05). The half-life of sulfated and nonsulfated gastrin-6 was 1.5 ± 0.4 and 1.4 ± 0.3 min, the metabolic clearance rate (MCR) was 80.8 ± 7.6 and 116.0 ± 13.5 ml·kg⁻¹·min⁻¹ (P < 0.05), and the apparent volume of distribution (Vdss) was 199.3 ± 70.1 and 231.4 ± 37.3 ml/kg, respectively. The decay of gastrin-52 in plasma was biexponential. The half-lives of this biexponential after a bolus injection were 3.9 ± 0.5 (T1/2α) and 25.7 ± 1.4 (T1/2β) min, and the MCR and Vdss were 4.2 ± 0.4 ml·kg⁻¹·min⁻¹ and 116.2 ± 16.2 ml/kg. We conclude that there is a differential elimination of progastrin products in splanchnic and nonsplanchnic tissue, which depends on the chain length of the peptides. Sulfation of gastrin-6 had no influence on the organ-specific extraction but reduced the MCR. Our results are in keeping with previous studies of nonsulfated gastrin-6 and the large nonsulfated gastrin-52, which are released in approximately equal amounts, and gastrin-71 and -52 each account for <1% (13, 27).

The metabolism and clearance from circulation of gastrin-34, -17, and -14 have been well studied in humans and other mammals (2, 4, 24, 31, 32, 34–36). Moreover, the metabolism of glycine-extended gastrin-17 has been thoroughly investigated (3, 24). Glycine-extended gastrins are the immediate precursors of the carboxyamidated active gastrins, and they are also released from G cells into circulation (13, 14). Under normal conditions, gastrin-17 and -34 are the predominant plasma gastrins in most mammals (25). However, as the molecular forms in plasma change toward longer or smaller, less processed forms in diseases with increased gastrin synthesis (16), it is relevant to examine the metabolism and kinetics of all circulating gastrin forms.

Gastrin-71, -52, and -6 are the most recently identified gastrins (12, 27, 29). So far, gastrin-71 has not been synthesized or purified in amounts sufficient for metabolic studies. In this study we have, therefore, examined only gastrin-52 and -6. The study was undertaken in anesthetized pigs during separate administration of each peptide, and the metabolic parameters were compared with those of gastrin-17 (24).

MATERIALS AND METHODS

Peptides

Sulfated gastrin-6 (mol wt 897 Da), nonsulfated gastrin-6 (mol wt 817 Da), and nonsulfated gastrin-52 (mol wt 6,018 Da) were custom synthesized by Cambridge Research Biochemicals (Zeneca, Cheshire, UK). Peptide content and purity were controlled in our laboratory by amino acid analysis (LKB amino acid analyzer with fluorescence detection; LKB Biochrom, Cambridge, UK) and reverse-phase HPLC (model 10843; Hewlett-Packard, Palo Alto, CA). Before infusion, the peptides were diluted in isotonic saline with 1 g/l of human albumin.

Gastrointestinal hormones

Gastrin is an important gastrointestinal hormone that regulates gastric acid secretion and the growth of gastric mucosal cells (for review, see Ref. 6). Gastrin is synthesized mainly in antral progastrin cells of the stomach. The cellular synthesis is a complex process, during which progastrin is processed to a number of bioactive gastrins (12, 27, 29). In antral venous blood, gastrin-17 accounts for >80% of postprandially released α-amidated gastrin. The remainder is mainly gastrin-34 and -6, which are released in approximately equal amounts, and gastrin-71 and -52 each account for <1% (13, 27).

The kinetics and metabolism in various organs of three bioactive products of progastrin, the small sulfated and nonsulfated gastrin-6 and the large nonsulfated gastrin-52, were examined during intravenous administration in anesthetized pigs. The kidney, hindlimb, liver, head, and gut eliminated the hexapeptides efficiently, with a fractional extraction ranging from 0.50 to 0.28 (P < 0.001–0.05). No metabolism was recorded in the lungs, and sulfation was without influence on the extraction of gastrin-6. Gastrin-52 was eliminated only in the kidney and the head, with a fractional extraction between 0.23 and 0.11 (P < 0.01–0.05). The half-life of sulfated and nonsulfated gastrin-6 was 1.5 ± 0.4 and 1.4 ± 0.3 min, the metabolic clearance rate (MCR) was 80.8 ± 7.6 and 116.0 ± 13.5 ml·kg⁻¹·min⁻¹ (P < 0.05), and the apparent volume of distribution (Vdss) was 199.3 ± 70.1 and 231.4 ± 37.3 ml/kg, respectively. The decay of gastrin-52 in plasma was biexponential. The half-lives of this biexponential after a bolus injection were 3.9 ± 0.5 (T1/2α) and 25.7 ± 1.4 (T1/2β) min, and the MCR and Vdss were 4.2 ± 0.4 ml·kg⁻¹·min⁻¹ and 116.2 ± 16.2 ml/kg. We conclude that there is a differential elimination of progastrin products in splanchnic and nonsplanchnic tissue, which depends on the chain length of the peptides. Sulfation of gastrin-6 had no influence on the organ-specific extraction but reduced the MCR. Our results are in keeping with previous studies of nonsulfated gastrin-6 and the large nonsulfated gastrin-52, which are released in approximately equal amounts, and gastrin-71 and -52 each account for <1% (13, 27).
Animal Preparations

The investigations were carried out in four groups of eight pigs (Danish Landrace-Yorkshire breed, 30–40 kg) under general anesthesia. The animals were fasted overnight but had free access to water. The study conformed with the legal requirements for animal experiments.

Anesthesia was introduced with ketamine (8 mg/kg Ketalar; Warner-Lambert/Parke Davis, Morris Plains, NJ) and a gas mixture of 1% halothane (Halocarbon Laboratories) and nitrous oxide in oxygen (2:1). After intubation, halothane was withdrawn and anesthesia was continued with repeated doses of pentobarbital sodium (2.5 mg/kg Mebumal; Nycomed DAK, Copenhagen, Denmark) and pancuronium (0.1 mg/kg Pavulon; Organon, Oss, The Netherlands) as a supplement to nitrous oxide under intermittent positive-pressure ventilation. Isotonic saline was infused at 10 ml·kg\(^{-1}\)·h\(^{-1}\) in all pigs during the whole experiment. In addition, donor blood from siblings was constantly infused with a pump in volumes equal to that removed during blood sampling. Arterial blood pressure and electrocardiograms were continuously monitored, and cardiac output was measured regularly with a Swan-Ganz thermodilution catheter (Swan-Ganz pediatric 5F; Baxter Health Care, Santa Ana, CA). Carbon dioxide tension, oxygen tension and saturation, pH, and standard bicarbonate were measured in all vascular beds used for blood sampling (ABL 2; Radiometer, Copenhagen, Denmark).

Catheters for blood sampling were placed as follows. Polyethylene catheters were positioned in the thoracic aorta via the left carotid artery and cephalically in the left internal jugular vein. Via the external jugular veins, two angiography catheters were introduced into a major hepatic vein and the left renal vein. Blood from a peripheral lung artery was drawn from the Swan-Ganz catheter. The positions of all catheters were controlled fluoroscopically. Neither the hepatic nor the pulmonary catheters were wedged, to avoid mixing with portal and pulmonary venous blood when samples were drawn. After a midline laparotomy, a catheter was introduced into the entrance of the portal vein via the splenic vein and another catheter was positioned in one of the major tributaries of the mesenteric vein draining the small intest...
tine. Blood from the femoral vein was drawn from an inserted catheter after exposure of the vessels in the groin. Peptides and indicator substance were infused into catheters with their tips placed in the right atria. After completion of surgery, a stabilization period of 1 h was allowed before start of the experiments.

Blood flow in the renal and femoral artery and the portal vein was measured with an electromagnetic flowmeter (Nyctron, Oslo, Norway), and pulmonary flow was regarded as equivalent to the cardiac output. Blood flow was converted to plasma flow from the hematocrit. Haptic plasma flow was measured by continuous infusion of indocyanine green (Cardiogreen; Becton Dickinson, Cockeysvile, MD) at 0.130 μmol/min. After a calibration period of 90 min, simultaneous samples from the aorta and the hepatic vein were drawn at 10-min intervals. The concentration of indocyanine in plasma was measured spectrophotometrically, and haptic plasma flow was calculated according to Fick’s principle as

\[
\text{Flow} = \frac{Q}{[0.05(BW) \times 0.5(dC_a/dt + dC_v/dt)]}
\]

where \(Q\) is dose rate, \(BW\) is body weight, \(C_a\) and \(C_v\) are plasma concentrations in the aorta and the hepatic vein, and \(dC_a/dt\) and \(dC_v/dt\) are the linear regressions of the arterial and venous concentrations with time (t).

**Experiments**

**Elimination of sulfated and nonsulfated gastrin-6.** The elimination of the peptides was measured from the concentration gradient across the vascular beds during separate infusion of sulfated gastrin-6 (460 pmol·kg \(^{-1}\)·h \(^{-1}\)) and nonsulfated gastrin-6 (600 pmol·kg \(^{-1}\)·h \(^{-1}\)) in two groups of eight pigs. The infusion rates were determined from previous pilot studies. Arterial samples were taken from the aorta every 15 min during infusion. After 90 min, venous samples were drawn every 15 min for 1 h from a lung artery and the femoral, renal, portal, hepatic, internal jugular, and cranial mesenteric veins. The cranial mesenteric vein is almost equivalent to the superior mesenteric vein in humans and drains the major part of the blood from the small intestine and the first part of the large intestine. When the infusion of the peptides was terminated after 170 min, aortic blood was sampled at regular intervals for 90 min to determine the half-life of the peptides.

**Elimination of gastrin-52.** Gastrin-52 was infused at 12 pmol·kg \(^{-1}\)·h \(^{-1}\) in eight pigs; the experimental design was otherwise similar to the study of gastrin-6. Because the disappearance of gastrin-52 from plasma follows a two-compartment model, the kinetic parameters were also calculated after a bolus injection (5 pmol·kg \(^{-1}\)·h \(^{-1}\)) in another group of eight pigs. Arterial blood samples were drawn at regular intervals until 90 min from the time of the injected bolus. In vitro metabolism and recovery of gastrin-6 and -52. The metabolism of peptides in plasma was evaluated by separate incubation of sulfated and nonsulfated gastrin-6 as well as gastrin-52 in porcine plasma at room temperature for 0, 1, 2, 4, 8, and 24 h. After incubation, the samples were immediately frozen in liquid nitrogen and stored at -20°C until analysis.

**Laboratory Analyses**

**Radioimmunoassay.** Blood samples were collected in ice-chilled tubes containing 50 IU heparin and 250 μl aprotinin (5,000 KIU) and placed on ice. After centrifugation, plasma was stored at -20°C until radioimmunoanalysis. All radioimmunoassay measurements were performed with previously described assays developed in our laboratory. All samples of each experiment were performed in a single assay.

Antiserum 2604 measures total concentration of carboxyamidated gastrins larger than the hexapeptides (30). The antiserum was raised against the α-amidated 2–17 fragment of human gastrin-17 and is directed against the COOH terminal part of the peptide. The antiserum binds gastrin-71, -52, -34, and -17 with equimolar potency; the reactivity with CCK is <0.5%. Synthetic human gastrin-17 was used as standard, and monooiodinated \(^{125}\)I-gastrin-17 was used as a tracer (26). The accuracy of the assay was evaluated by the recovery of known amounts of peptide added to plasma. The precision (intra-assay variation) was expressed by the coefficient of variation of repeated measurements of peptide in plasma at three different concentrations. The accuracy ranged from 84 to 102%, and the precision was 5%.

Sulfated and nonsulfated gastrin-6 were measured with antiserum 2609 after extraction of the peptides from plasma. Antiserum 2609 was also raised against the α-amidated 2–17 fragment of human nonsulfated gastrin-17 (28). In contrast to antiserum 2604, which requires an epitope of seven residues or more for binding, antiserum 2609 requires only the COOH terminal tetrapeptide amide sequence for binding. The reactivity is 63% with gastrin-34 and 20% with CCK-8. One milliliter of plasma was extracted with 2 ml of 96% ethanol, and the supernatant was evaporated under a constant air flow. The dry extracts were then reconstituted in assay buffer. Sulfated and nonsulfated gastrin-6 were used as standards, and monooiodinated \(^{125}\)I-gastrin-17 was used as a tracer. Measurements of sulfated gastrin-6 had an accuracy of 91–98% and a precision of 1–8%. For nonsulfated gastrin-6, the accuracy ranged from 86 to 95% and the precision ranged from 4 to 6%.

Gastrin-52 was measured using antiserum 88235, which was raised against fragment 20–33 of human progastrin that corresponds to the NH₂ terminus of gastrin-52 (29). The antiserum is specific for the sequence 20–25 of progastrin. The antiserum does not bind gastrin-34, -34, or smaller carboxyamidated gastrins, nor does it react with CCK. Synthetic human gastrin-52 was used as standard, and monooiodinated \(^{125}\)I-gastrin-52 was used as a tracer (26). The accuracy of measurements ranged from 80 to 94%, and the precision was 20%.

**Chromatography.** The elution profile of each peptide was determined by means of gel chromatography. Plasma from eight pigs of each group was pooled, and 1-ml samples were applied to Sephadex G-50 superfine columns (10,000 mm and 12 × 2,000 mm; Pharmacia, Uppsala, Sweden). The short columns were eluted with 0.125 M NH₄HCO₃ (pH 8.2) with a flow rate of 4 ml/h and calibrated with sulfated and nonsulfated gastrin-6. The long columns were eluted like the short columns but with a flow rate of 3 ml/h and calibrated with gastrin-52. Void volume and total volume were determined by elution of \(^{125}\)I-albumin and \(^{22}\)NaCl. Fractions of 1 ml were obtained and analyzed with antisera 2604, 2609, and 88235. The elution position (\(K_d\)) was calculated as

\[
K_d = (V_e - V_v)/(V_i - V_v)
\]

where \(V_e\) is the elution volume of the peptides and \(V_v\) and \(V_i\) are elution volumes of \(^{125}\)I-albumin and \(^{22}\)NaCl, respectively.

**Calculations**

The fractional extraction of the peptides in the vascular beds was calculated as

\[
E_R = (C_i - C_v)/C_i
\]
where $C_i$ and $C_o$ are inflow and outflow concentrations in plasma. The fractional extraction in the liver was grossly estimated from the difference between the inflow concentrations in arterial ($C_p$) and portal ($C_p$') plasma and the outflow concentration in hepatic venous plasma ($C_h$) and the respective flows ($F$)

$$E_{(h/p)} = \frac{(F_p C_p + F_{p'} C_{p'}) - F_h C_h}{(F_p C_p + F_{p'} C_{p'})} = 1 - \frac{F_h C_h}{(F_p C_p + F_{p'} C_{p'})}$$

Arterial flow was estimated by subtraction of portal flow from total liver flow. Clearance of the peptides at the metabolic sites was calculated by multiplying extraction ratio with plasma flow.

The kinetics of sulfated and nonsulfated gastrin-6 were calculated according to a one-compartment open model

$$C_t = C_0e^{-k_t t}$$

where $C_0$ and $C_t$ are plasma concentrations at zero time and $t$. The half-life was determined from the elimination in arterial plasma after termination of the infusion. Plasma concentrations were plotted on semilogarithmic graph paper after subtraction of basal values. Linear regression of the logarithm of plasma concentration vs. time was computed to yield the slope ($k_t$), from which the half-life was calculated by dividing into 0.693. The metabolic clearance rate (MCR), which is total body clearance, was calculated from dose rate divided by the plateau increment of peptide in plasma. The apparent volume of distribution ($V_d$) was determined by dividing MCR with $k_t$ of the regression line for disappearance.

The kinetics of gastrin-52 were analyzed according to a two-compartment open model

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where $A$ and $B$ are the zero intercepts with the ordinate of the individual exponential terms and $\alpha$ and $\beta$ are the slopes of the curves. When a substance displays two- or multicompartment kinetics, prolonged infusion may load the deep compartment with the substance, so that the postinfusional plasma curve shows little distributional activity and is of limited analytic value. Therefore, the parameters were calculated from postinfusional data and from the decay in plasma after a bolus injection. When model parameters were calculated from postinfusional data, the values of $A$ and $B$, which would have followed a single intravenous dose of the same magnitude, were calculated from the zero intercepts $R$ and $S$ at the start of the postinfusional period

$$A = R \frac{\alpha T}{(1 - e^{-\alpha T})} \quad B = S \frac{\beta T}{(1 - e^{-\beta T})}$$

where $T$ is the period of infusion (18). Area under the curve (AUC) was determined as

$$\int_a^\infty C \, dt = (A/\alpha + B/\beta)$$

During constant-rate infusion, the MCR was calculated the same way as for gastrin-6. After bolus injection, MCR was calculated from injected dose divided by AUC. The apparent volume of distribution at steady state ($V_{des}$) was calculated as the sum of the central and peripheral compartment volumes. The rate constants of the model and the volumes of the central and peripheral compartments ($V_1$ and $V_2$) were calculated as

$$k_{21} = (A\beta + B\alpha)/(A + B) \quad k_{10} = \alpha/\beta k_{21} \quad k_{12} = \alpha + \beta - k_{10} - k_{21}$$

$$V_1 = \text{dose}/(A + B) \quad V_2 = V_1 k_{12}/k_{21}$$

where $k_{12}$ and $k_{21}$ are the first-order rate constants of distribution between the central and peripheral compartment and $k_{10}$ is the sum of the simultaneous processes of metabolism and excretion.

All results are expressed as means ± SE. Statistical analysis was performed with Wilcoxon’s test for paired samples and the Mann-Whitney test for unpaired samples. $P$ values < 0.05 were considered significant.

**RESULTS**

**Elimination of Sulfated and Nonsulfated Gastrin-6**

The concentrations of the peptides during the studies are shown in Fig. 2. The corresponding dose rates of sulfated and nonsulfated gastrin-6 estimated from the concentrations in the infusion lines were 462.0 ± 20.4 and 597.9 ± 53.5 pmol·kg⁻¹·h⁻¹, respectively.

Extraction of sulfated gastrin-6 was recorded over the kidney ($P < 0.01$), liver ($P < 0.01$), gut ($P < 0.01$), hindlimb ($P < 0.01$), and head ($P < 0.01$), whereas pulmonary extraction was not significant (Fig. 3; Table 1). Extraction of nonsulfated gastrin-6 was observed over the kidney ($P < 0.01$), liver ($P < 0.05$), gut ($P < 0.01$), hindlimb ($P < 0.01$), and head ($P < 0.01$) but not over the lungs (Fig. 4; Table 1). The fractional extraction of sulfated and nonsulfated gastrin-6 was not significantly different.

The kinetics of sulfated and nonsulfated gastrin-6 followed a one-compartment open model (Fig. 5). The MCR, half-life, and $V_d$ of sulfated gastrin-6 were 80.8 ± 0.3 min (not significant), and 231.4 ± 37.3 ml/kg, respectively. For nonsulfated gastrin-6, the values amounted to 116.0 ± 13.5 ml·kg⁻¹·h⁻¹ (P < 0.05), 1.5 ± 0.4 min, and 199.3 ± 70.1 ml/kg, respectively. For nonsulfated gastrin-6, the values measured during the experiments and the calculated clearance of the peptides at the metabolic sites are presented in Tables 2 and 3.

![Fig. 2. Concentration (means ± SE) in arterial plasma during and after infusion of sulfated gastrin-6 (460 pmol·kg⁻¹·h⁻¹) and nonsulfated gastrin-6 (600 pmol·kg⁻¹·h⁻¹) in 2 groups of 8 pigs.](http://ajpgi.physiology.org/Downloadedfrom)
Elimination of Gastrin-52

Plasma concentrations of gastrin-52 during the study are shown in Fig. 6. The corresponding dose rate of the peptides was $11.7 \pm 1.7 \text{ pmol/kg} \cdot \text{h}$. The fractional extraction of gastrin-52 was significant in kidney ($P < 0.01$) and head ($P < 0.05$) but nonsignificant in the liver, gut, hindlimb, and lung (Fig. 7; Table 1). The extraction in kidney and head was lower than of sulfated and nonsulfated gastrin-6 ($P < 0.01$ and 0.05, respectively).

The kinetics of gastrin-52 followed a two-compartment open model (Fig. 8). When the pharmacokinetic parameters were calculated from postinfusional data, the half-lives of the biexponential, $T_{1/2a}$ and $T_{1/2b}$, were $4.3 \pm 0.8$ and $29.3 \pm 3.1$ min, and the MCR and $V_{dss}$ amounted to $3.1 \pm 0.2 \text{ ml/kg} \cdot \text{min}$ and $98.5 \pm 9.4 \text{ ml/kg}$, respectively. When the pharmacokinetics were calculated from plasma concentrations after a bolus injection ($5.3 \pm 0.2 \text{ pmol/kg}$), the half-lives were $3.9 \pm 0.5$ ($T_{1/2a}$) and $25.7 \pm 1.4$ min ($T_{1/2b}$), and MCR and $V_{dss}$ amounted to $4.2 \pm 0.4 \text{ ml/kg} \cdot \text{min}^{-1}$ and $116.2 \pm 16.2 \text{ ml/kg}$, respectively. The kinetics of the two peptides were not significantly different. The respective fractions of elimination during the $b$-phase, \((B/b)/AUC\), were 0.75 and 0.71, which implies that the major part of the elimination took place during the $b$-phase. Hemodynamic values measured during the investigations and the calculated clearance of the peptides at the metabolic sites are shown in Tables 1 and 2.

In Vitro Degradation of Progastrin Products

The recovery of sulfated and nonsulfated gastrin-6 during the first 8 h of incubation in plasma ranged from 90 to 97%. Gastrin-52 was stable for 4 h when immunoreactivity was measured with the NH$_2$ antibody. After 8 and 24 h, the immunoreactivity in plasma had decreased to 85 and 68%, respectively. There was no reduction in COOH terminal immunoreactivity during 24 h of incubation.

Chromatography

Gel chromatography of arterial plasma during infusion of sulfated and nonsulfated gastrin-6 revealed a peak at $K_d$ of 1.26 and 1.12, which corresponds to the elution positions of the standard calibration peptides (Fig. 9). Chromatography of gastrin-52 in arterial plasma revealed one major peak at a $K_d$ of 0.29, which is the elution position of the intact peptide. This peak was visible with the NH$_2$ terminal (antiserum 88235) and the COOH terminal antibody (antiserum 2604).

Table 1. Fractional extraction of progastrin products in the vascular beds of pigs

<table>
<thead>
<tr>
<th>Product</th>
<th>Kidney</th>
<th>Liver</th>
<th>Gut</th>
<th>Head</th>
<th>Hindlimb</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfated Gastrin-6</td>
<td>0.50 ± 0.03*</td>
<td>0.42 ± 0.06*</td>
<td>0.28 ± 0.05*</td>
<td>0.36 ± 0.07*</td>
<td>0.50 ± 0.05*</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Nonsulfated Gastrin-6</td>
<td>0.44 ± 0.05*</td>
<td>0.34 ± 0.06*</td>
<td>0.23 ± 0.03*</td>
<td>0.44 ± 0.08*</td>
<td>0.42 ± 0.05*</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Gastrin-52</td>
<td>0.23 ± 0.05*</td>
<td>0.05 ± 0.03</td>
<td>0.06 ± 0.04</td>
<td>0.16 ± 0.05*</td>
<td>0.08 ± 0.05</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Gastrin-17</td>
<td>0.40 ± 0.04*</td>
<td>0.07 ± 0.03</td>
<td>0.13 ± 0.03*</td>
<td>0.32 ± 0.04*</td>
<td>0.42 ± 0.04*</td>
<td>0.01 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8$; *$P < 0.01$; †$P < 0.05$. Data on gastrin-17 are from Palnæs Hansen et al. (24).
(Fig. 9). Smaller NH₂ or COOH terminal fragments were measured neither in arterial nor in venous plasma from the metabolically active sites in head and kidney.

DISCUSSION

The present study revealed a differential elimination of bioactive gastrins in organs as well as in nonsplanchnic tissue. Previous studies have shown that the major biosynthetic endproduct of progastrin, gastrin-17, is eliminated at multiple sites in the body (4, 24, 32). These observations led to the conclusion that gastrin is cleared in all major vascular beds. However, the present results suggest that this conclusion needs modification, since we found that the metabolic sites for bioactive gastrin varied according to the molecular size of the peptides. A differential metabolism of gastrin as well as other gastrointestinal peptides has so far only been recorded in the liver (7, 8, 34), but the present study also demonstrated a differential extraction in the gut and hindlimb. Only the head and kidneys were capable of eliminating gastrin peptides independent of their size but with a higher fractional extraction for small peptides.

An arteriovenous concentration gradient has been measured in the heads of different animals during infusion of gastrin-17 (4, 20, 32). In pigs, blood from the brain and the musculoskeletal part of the head have a common drainage into the internal jugular vein. Although selective venous samples from the brain were not obtained in this study, two observations are in favor of a cerebral gastrin metabolism. One is an abundant expression of the gastrin/CCKB receptor in the brain (17). The other is the selective extraction of the hexapeptides and gastrin-52 in the musculoskeletal system (the hindlimb), whereas gastrin-6 and -52 were both extracted in the head.

The kidneys have been regarded as important sites of extraction of circulating peptides. The glomerular filtration of gastrin is influenced by the Donnan effect, with an enhanced filtration of negatively charged peptides (21, 23, 33). After glomerular filtration, the peptides undergo an almost complete tubular absorption and metabolism, since excretion of intact peptides in urine accounts for <1% of the filtered amount. Since most gastrins, including gastrin-34, -17, and -6, are eliminated at several sites in the body with an integrated clearance exceeding that of the kidneys, renal contribution to the MCR seems only to be of major importance for large gastrins like gastrin-52 and, probably, gastrin-71. However, hypergastrinemia found in some patients with renal failure is only partially explained by low renal clearance of gastrin. An increased release of gastrin caused by hypo- or achlorhydria and hypercalcemia in combination with a decreased metabolism in other vascular beds are more likely explanations. Moreover, hemodialysis may contribute to the complexity of gastrin physiology in these patients (20).

### Table 2. Hemodynamics in 3 groups of 8 pigs during infusion of progastrin products

<table>
<thead>
<tr>
<th>Sulfated Gastrin-6</th>
<th>Nonsulfated Gastrin-6</th>
<th>Gastrin-52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, ml plasma/min</td>
<td>2,137 ± 208</td>
<td>2,205 ± 93</td>
</tr>
<tr>
<td>Plasma flow, ml/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral artery</td>
<td>81 ± 8</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>Renal artery</td>
<td>196 ± 17</td>
<td>145 ± 23</td>
</tr>
<tr>
<td>Portal vein</td>
<td>537 ± 24</td>
<td>833 ± 84</td>
</tr>
<tr>
<td>Liver</td>
<td>745 ± 25</td>
<td>963 ± 84</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>111 ± 3</td>
<td>111 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE.
The role of the liver in the metabolism of gastrointestinal peptides has been debated because of the number of conflicting results. Animal experiments in pig, dog, and rat with infusion of gastrin and CCK fragments of different chain length have shown that peptides with eight or fewer amino acid residues are subject to hepatic extraction, whereas larger peptides are increasingly resistant to elimination in the liver (5, 7, 8, 34). The present results confirm these observations, since only the hexapeptides were cleared in the liver. Earlier investigations by us (24) failed to demonstrate elimination of human gastrin-17 in the porcine liver, but hepatic elimination of postprandially released gastrin-17 has been recorded in pigs in two independent studies (3, 22). Since human gastrin-17 has been used in most animal studies of gastrin, the conflicting results could result from the species difference of the peptide. Despite the close structural similarity of gastrin-17 among mammalian species, only gastrin-6 is identical in animals and man, whereas larger gastrins differ with increasing chain length. Studies of the CCK octapeptide, which bears a close resemblance to gastrin-6, have shown that hepatic extraction is sensitive to even minor modifications of the molecule (8).

Besides the liver, both the gut and hindlimb displayed a differential elimination of gastrin with extraction of the hexapeptides, whereas gastrin-52 passed unhindered. Since clearance of gastrin-17 has also been recorded in the hindlimb of pigs (24), the musculoskeletal system, therefore, represents the major metabolic site of short- and medium-sized gastrin peptides. The lungs were the only site without a significant gastrin elimination, and earlier studies in pigs also failed to demonstrate pulmonary extraction of gastrin-17.

![Fig. 6. Concentration (means ± SE) of gastrin-52 in arterial plasma during and after infusion of the peptide (12 pmol·kg⁻¹·h⁻¹) in 8 pigs with the use of antiserum 88235.](image)

![Fig. 7. Plasma concentrations (means ± SE) of gastrin-52 during infusion of the peptide (12 pmol·kg⁻¹·h⁻¹) in 8 pigs with the use of antiserum 88235.](image)

![Fig. 8. Elimination of gastrin-52 in arterial plasma after termination of constant-rate infusion (12 pmol·kg⁻¹·h⁻¹; A) and after bolus injection (5 pmol/kg; B) in 2 groups of 8 pigs. The 2-compartment model calculated from postinfusional data had volumes of central (V₁) and peripheral (V₂) compartments, respectively, of 46.6 ± 6.6 and 51.9 ± 12.1 ml/kg, and rate constants of k₁₂ = 0.098 ± 0.034, k₂₁ = 0.068 ± 0.006, and k₁₀ = 0.073 ± 0.012 pmol/min. The respective values after a bolus injection were V₁ = 57.2 ± 6.0 and V₂ = 59.0 ± 12.5 ml/kg and k₁₂ = 0.076 ± 0.012, k₂₁ = 0.073 ± 0.009, and k₁₀ = 0.075 ± 0.005 pmol/min. In this instance, k₁₂ and k₂₁ are the first-order rate constants of distribution between the central and peripheral compartments, and k₁₀ is the sum of the simultaneous processes of metabolism and excretion.](image)
17. However, a small pulmonary extraction of gastrin-17 of ~10% has been measured in sheep (4). This means that the lungs in shear are responsible for the metabolism of as much as 50% of circulating gastrin and, therefore, represent the major metabolic organ in gastrin metabolism. However, even small variations in the measurement of venous and arterial concentrations may lead to a false high clearance due to the high pulmonary blood flow.

Degradation of the peptides in vitro was slow, since gastrin-52 and -6 retained their immunoreactivity for several hours after incubation in plasma. Metabolic products of gastrin-52 in plasma were recorded neither in vitro nor in vivo, probably due to low concentrations of the intact peptide.

Derivatization of gastrin peptides increases their resistance to metabolism. Thus amidation of the COOH terminal carboxy group protects against carboxypeptidases. This was evident from incubation of gastrin-52 in plasma, which resulted in a decreased NH2 terminal immunoreactivity, whereas the COOH immunoreactivity was retained after 24 h. Also, sulfation of gastrin enhances resistance to metabolism, but this may only apply to small peptides. In humans, the MCRs of sulfated and nonsulfated gastrin-17 were found to be approximately the same (1), whereas MCR of sulfated hexagastrin in cat was nearly sixfold higher than the nonsulfated form (15). In the present study, sulfated gastrin-6 had a 30% lower MCR than the nonsulfated form. Apart from derivatizations, the MCR also depends on the chain length of the peptides. Thus the higher MCR of gastrin-6 compared with gastrin-52 is in accordance with the general experience that clearance of peptides varies inversely with chain length. There was a good agreement between the MCR of gastrin-52 and the integrated organ clearance, but a considerable difference was recorded for gastrin-6, also when an estimation for the clearance by the trunk was made. A similar discrepancy was present in metabolic studies of the octapeptide of CCK in pigs (5). A false high value of the MCR may result from either intravascular degradation or from sequestration of the small peptides at extravascular sites. This would also lead to a high Vd, which for sulfated and nonsulfated gastrin-6 was twice as high as Vd for gastrin-52 and -17 (24).

We conclude that the differential elimination of gastrin found in pigs is related to chain length of the peptide. A selective elimination was recorded in most vascular beds. Only the kidney and the brain displayed a nonselective extraction of gastrin.

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