Serum level of immunoreactive gastrin: influence of kidney function¹

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SUMMARY Serum gastrin levels have been studied in 70 patients with chronically reduced glomerular filtration rate (GFR) as estimated by ⁵¹Cr-EDTA clearance, creatinine, and β_2 -microglobulin values. A strong dependence upon GFR was found, although the correlation between gastrin levels and GFR was not as high as that between β_2 -microglobulin and GFR, indicating the existence of extrarenal factors regulating the levels of circulating gastrin. In a separate group of 31 patients on maintenance dialysis the mean gastrin level was 65.9 pmol/l—that is, a fourfold increase compared to healthy subjects. Three of the uraemic patients had pronounced rises in serum gastrin in the range 800-1800 pmol/l. Finally, the influence of acute alterations of kidney function on serum gastrin was studied in 11 patients undergoing renal transplantation. In addition to a GFR dependence the results indicate the existence of feedback mechanisms in gastrin homeostasis. Although the clinical importance of the increased gastrin levels in renal failure is unknown, hypergastrinaemia occurs with sufficient frequency to be involved in upper gastrointestinal complications of uraemic patients.

Studies on the metabolism of gastrin in various experimental systems suggest that the kidneys are the major site of gastrin degradation (Newton and Jaffe, 1971; Booth *et al.*, 1973; Clendinnen *et al.*, 1973; Davidson *et al.*, 1974; El Munshid *et al.*, 1976). Additional or alternative routes of excretion and catabolism have also been suggested (Becker *et al.*, 1973). The kidneys are known to play a central role in the regulation and metabolism of low molecular weight proteins (LMW) such as free immunoglobulin light chains, β_2 -microglobulin (Berggård and Bearn, 1968), and protein hormones (Maack, 1975). β_2 -microglobulin (mol weight 11.800) constitutes a constant part of the cell membrane-bound histocompatibility antigens (Nakamuro *et al.*, 1973; Peterson *et al.*, 1974) and has been shown to be strongly dependent on the glomerular filtration rate for its elimination from plasma (Wibell *et al.*, 1973). Filtered β_2 -microglobulin is normally reabsorbed and catabolised within the renal proximal tubules.

There seems to be no significant feedback regulation of the serum concentration of β_2 -microglobulin and the extrarenal elimination of the protein is apparently small. These properties make β_2 -microglobulin a suitable marker for comparison with other LMW-proteins, such as gastrin, in the evaluation of the renal role in controlling the serum level of this polypeptide hormone.

In this study, the influence of the glomerular filtration rate (GFR) on the serum levels of gastrin and of β_2 -microglobulin have been investigated in a group of patients with renal disorders that had been investigated by ⁵¹Cr-EDTA clearance. The correlation between this clearance estimate and the serum levels of the proteins is compared and discussed. Also a group of patients with longstanding uraemia and severely reduced GFR was studied by analysis of serum creatinine, gastrin, and β_2 -microglobulin. The serum level of gastrin was studied in 11 patients undergoing renal transplantation in order to obtain information about regulatory mechanisms other than the glomerular filtration rate.

Methods

PATIENTS

Patients from the University Hospital of Uppsala for whom a ⁵¹Cr-EDTA clearance had been requested

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were studied. The study included 70 consecutive patients, of whom 40 were males. The mean age was 41 (range 16-71) years. The patients had the following diagnoses: two and three years follow-up after renal transplantation (18 cases), glomerulonephritis (15 cases), artificial urinary bladder of Bricker type (11 cases), hydronephrosis (eight cases), pyelonephritis (eight cases), unilateral nephrectomy due to hypernephroma (four cases), diabetic nephropathy (two cases), renal congenital dysplasia (two cases), and SLE (two cases).

Observations were also made on 31 other patients with uraemia and on chronic maintenance haemodialysis, of whom 18 were males. The mean age was 38 (range 18-54) years. The dominating renal disorder of these patients was glomerulonephritis. Eleven of these patients were transplanted with a cadaver kidney.

ENDOGENOUS CREATININE CLEARANCE

Creatinine was determined in serum and urine by autoanalyser technique (Technicon N 11b). Creatinine clearance was calculated from a clearance period of 24 hours. The variation coefficient of the creatinine method was 2%.

⁵¹CR-EDTA-CLEARANCE

The trace substance was obtained from Hoechst, W. Germany. The single injection technique as described by Bröchner-Mortensen (1974) was used. The plasma concentrations were used to calculate the plasma clearance of ⁵¹Cr-EDTA according to the equation:

$$Cl_1 = \frac{Q}{C \cdot e^{-b \cdot dt}}$$

Q represents the administered dose and the denominator the area under the plasma concentration curve. The clearance was corrected according to the equation $Cl = 0.990778 Cl_1 - 0.01218 Cl_1^2$, and finally to 1.73 m² body surface.

RADIOIMMUNOCHEMISTRY

Fasting blood samples were drawn aseptically into plain tubes and serum was stored at -20°C until analysis in the same analytical series. β_2 -microglobulin was determined by a radioimmunoassay kit, Phadebas β_2 -micro test, purchased from Pharmacia, Uppsala, Sweden.

Serum gastrin was measured by a radioimmunosorbent assay (Lundqvist and Wide 1977). The anti-gastrin serum (2604) was a generous gift from Professor J. Rehfeld, Aarhus, Denmark. Synthetic human gastrin I (ICI Chemicals, England) was used for iodination and as standards in the assay. The

results were expressed as pmol equivalents of SHG I per litre.

In order to study the possible interaction of non-gastrin substances in the gastrin assay, serum dilution curves were constructed and compared to the standard curve.

The intra-assay variation for β_2 -microglobulin was 6% and for gastrin 5.4%.

GEL CHROMATOGRAPHY

Fractionation of 3 ml serum samples from three patients with uraemia and hypergastrinaemia was performed by gel chromatography on a 90 × 2.6 cm column of Biogel P10 at +4°C in 0.01 M Tris hydrochloride buffer, pH 7.5. The flow rate was 10 ml/h. The column was calibrated with ¹²⁵I-insulin and Na¹²⁵I. The void fractions were detected by measuring optical density at 280 nm.

Results

⁵¹CR-EDTA-CLEARANCE AND THE SERUM LEVELS OF GASTRIN AND β_2 -MICROGLOBULIN

By the use of double logarithmic scales, linear regression to the glomerular filtration rate (measured as clearance of ⁵¹Cr-EDTA) was calculated for the serum level of β_2 -microglobulin and the serum level of gastrin. There was a strong correlation between log β_2 -microglobulin/serum and log ⁵¹Cr-EDTA clearance ($r = 0.93$; $P < 0.001$). The gastrin level correlated with the GFR although to a lesser extent ($r = 0.45$; $P < 0.001$) as shown in Fig. 1. The correlation coefficient between log β_2 -microglobulin/serum and log gastrin/serum was 0.44 (Fig. 2). These findings suggest that extrarenal factors are of importance and influence the plasma concentration of immunoreactive gastrin.

SERUM LEVELS OF GASTRIN AND β_2 -MICROGLOBULIN IN DIALYSIS PATIENTS

Pre-dialysis serum gastrin and β_2 -microglobulin levels were assayed in patients on chronic maintenance haemodialysis. The endogenous creatinine clearance was less than 3 ml/min in each patient. The geometric median serum gastrin level in this group of patients was slightly but significantly ($P < 0.001$) raised (65.0 pmol/l), when compared to the reference value obtained in our laboratory (16.8 pmol/l, 4-62 (2 SD)) pmol/l. It should, however, be noticed that several patients with this low filtration rate show gastrin levels within the normal range (Fig. 3). On the other hand, the serum levels of β_2 -microglobulin in patients with uraemia (median value 28.5), were markedly raised compared to those of healthy subjects (median value 1.6 mg/l, 1.1-2.4 mg/l (2 SD)).

Fig. 1 The serum level of gastrin versus ^{51}Cr -EDTA clearance in 70 patients with renal disease. The solid line is the regression line ($\log y = -0.345 \log x + 2.252$). The correlation coefficient is 0.45 ($P < 0.001$).

Fig. 2 Correlation between the serum level of gastrin and β_2 -microglobulin in 70 patients with renal disease. The solid line is the regression line ($\log y = 0.353 \log x - 0.071$). The correlation coefficient is 0.44 ($P < 0.001$).

Three patients with uraemia had especially high gastrin levels. Their sera were further analysed in different dilutions in order to study any possible interaction of other substances in the assay. The

serum dilution curves demonstrated a parallel course when compared to the standard curve, as illustrated in Fig. 4, indicating an adequate specificity of the determinations. Gel chromatography of these sera was carried out and the different fractions were assayed for immunoreactive gastrin. The activity patterns obtained (exemplified by one patient in Fig. 5), showed the presence of components I-IV with component II as the predominating form (51-62%) of immunoreactive gastrin in these cases.

Fig. 3 Basal serum gastrin (●) and β_2 -microglobulin (○) levels in 31 patients on maintenance haemodialysis. The geometric median values ± 2 SD of gastrin and β_2 -microglobulin are indicated. The reference range of these substances in sera of apparently healthy individuals are also shown.

In a second part of this study, in 11 patients transplanted with a kidney graft, we correlated the time course and magnitude of gastrinaemia with two variables of GFR, serum β_2 -microglobulin and serum creatinine. Two different patterns of variation in gastrin levels could be distinguished in these patients. In seven cases in which serum gastrin concentration before transplantation was high or moderately raised, an initial fall in serum gastrin after transplantation was evident (Fig. 6a). On the other hand, when the serum level of gastrin at the time of transplantation was within the normal

Fig. 4 Dilution curve from serum of a patient with hypergastrinaemia and severe renal failure.

range (four cases), no significant reduction of the gastrin level was observed during the days after the implantation (Fig. 6b).

Increases in gastrin levels were found in all cases that had a transient reduction in GFR because of an acute rejection episode. In three of the transplanted patients, rejection episodes gave rise to persistently impaired GFR. In these cases, the serum β_2 -microglobulin and creatinine levels remained at constantly raised levels. After an initial increase, the gastrin levels gradually declined over a period of 10-25 days to the level before rejection. This phenomenon is illustrated by one patient in Fig. 6c.

Discussion

The good correlation between ^{51}Cr -EDTA clearance and serum β_2 -microglobulin levels and the rapid decline in β_2 -microglobulin levels early after renal transplantation confirms the strong influence of GFR for β_2 -microglobulin concentrations that has been previously published (Wibell *et al.*, 1973). A decrease in β_2 -microglobulin concentrations has been reported after transplantation even before urine production has become significant and is thought to be due to a functional catabolic activity of the kidneys independent of urine formation (Bernier and Post, 1973). In our patients, urine output occurred early after transplantation and no obvious dissociation in

Fig. 5 Fractionation pattern of serum from a patient with severe renal failure on a Biogel P-10 column with optical density (OD) measurement at 280 nm (solid line). The column was calibrated with ^{125}I -insulin and Na^{125}I which are indicated in the figure. Each fraction of 3 ml was assayed for gastrin (●—●).

Fig. 6 Serial studies of serum levels of creatinine, β_2 -microglobulin and gastrin on three patients after renal transplantation. Start of rejection episodes is indicated by arrows. (A) A patient with hypergastrinaemia before transplantation. (B) Variation of serum gastrin levels in a patient with normal gastrin levels before transplantation of kidney grafts. (C) Pattern of gastrinaemia after a rejection episode resulting in constantly impaired GFR.

time between creatinine and β_2 -microglobulin levels was noticed.

When the results of gastrin levels in this study are compared with β_2 -microglobulin values, the following observations were made. Eventually, along with rapid improvement of the GFR, such as occurred after a successful renal transplantation, the serum gastrin behaved similarly to β_2 -microglobulin. Exceptions were the patients who had normal gastrin levels at the time of transplantation. In these cases, no decline in gastrin levels occurred. Secondly, in patients with longstanding renal insufficiency—that is, the group investigated by ^{51}Cr -EDTA clearance and the group of dialysis patients—the correlation obtained between GFR and gastrin levels was more modest than that for GFR and β_2 -microglobulin, which suggests that in such cases the serum gastrin level is also modulated by extrarenal factors.

Shifts in gastrin levels when GFR is rapidly changed have been described earlier. Decrements in serum gastrin were noted in patients after improve-

ment in renal function (Korman *et al.*, 1972; King and Hansky, 1974). An increase in serum gastrin in a few cases of rejection after renal transplantation has also been noted (Hansky *et al.*, 1975). These observations agree with our data on patients undergoing transplantation.

In situations with chronically reduced glomerular filtration, reports of serum gastrin have been sparse, and results have been interpreted in a contradictory way. Reeder and Thompson (1971) studied five patients with uraemia and found normal values of gastrin before and after haemodialysis. In contrast, increased serum gastrin concentrations have been reported in patients with severe chronic renal failure (Korman *et al.*, 1972; Hansky *et al.*, 1975) and also in nephrectomised patients (Maxwell *et al.*, 1971). These seemingly contradictory data could be explained by the large variation from normal to highly raised levels of serum gastrin in patients with advanced uraemia noticed in the present study.

The serum level of gastrin is determined by

synthesis rate, release into, and elimination of gastrin from the circulation. Our observation of the wide range of serum gastrin in patients on dialysis could be explained not only by a renal role in gastrin catabolism, but might also indicate a feedback mechanism leading to a decreased synthesis and/or release of gastrin from the gastrin producing cells when the renal elimination of the hormone is impaired. In fact, such a hypothesis has previously been suggested by Durkin *et al.* (1971). The existence of such regulatory mechanisms is also indicated by the results from serial observations of serum gastrin on three patients with rejection of their kidney grafts. A steady decline of gastrin levels was noticed in these cases over a period of 10 to 25 days, although the GFR remained constantly reduced. The results might be interpreted to mean that mechanisms for gastrin homeostasis operate during this period of time. In other cases with sudden impairment of renal function, regulation of gastrin production might act in a slower and less efficient manner leading to constantly raised levels of the hormone in the circulation. The normal regulation of gastrin synthesis is less known, but previous studies by Creutzfeldt *et al.* (1971) and others indicate a direct effect of hydrochloric acid. The synthesis rate of gastrin in renal insufficiency might also be modulated by such a mechanism. There is also the possibility of alternative routes of excretion and catabolism of gastrin which might regulate the serum level of gastrin in uraemia even if the synthesis is unaffected.

In this study, 10% of the patients on maintenance dialysis had gastrin levels in the range normally associated with the Zollinger-Ellison syndrome. In these individuals, there might be a change in the proportions of different molecular forms of gastrin towards larger molecules with an increase in half-life leading to raised serum levels. In fact, such a change in molecular distribution towards larger forms has been reported for plasma glucagon in uraemic patients (Kuku *et al.*, 1976). This possibility seems unlikely as the sera of dialysis patients showed an elution pattern for gastrin after gel filtration similar to that seen in healthy individuals (Rehfeld, 1972) with gastrin-34 being the predominant component.

The clinical importance and the biological relevance of the increased serum gastrin levels in renal failure are uncertain with the lack of information concerning gastric secretion and gastric mucosa histological structure. However, hypergastrinaemia occurs with sufficient frequency to be involved in the development of upper gastrointestinal ulceration in uraemia. A pathophysiological relationship of transient hypergastrinaemia observed in episodes of renal rejection and the high incidence of gastric

ulceration and bleeding in patients after kidney transplantation is a possibility. To enable a clear message to be given about this issue serial changes in serum gastrin levels have to be compared with repeated analyses of gastric acid secretion in patients with acute renal failure.

References

- Becker, H. D., Reeder, D. D., and Thompson, J. C. (1973). Extraction of circulating endogenous gastrin by the small bowel. *Gastroenterology*, **65**, 903-906.
- Berggård, I., and Bearn, A. G. (1968). Isolation and properties of a low molecular weight β_2 -globulin occurring in human biological fluids. *Journal of Biological Chemistry*, **243**, 4095-4103.
- Bernier, G. M., and Post, R. S. (1973). β_2 -microglobulin. A marker of renal homograft survival. *Transplantation*, **15**, 176-179.
- Booth, R. A. D., Reeder, D. D., Hjelmquist, U. B., Brandt, E. N., Jr, and Thompson, J. C. (1973). Renal inactivation of endogenous gastrin in dogs. *Archives of Surgery*, **106**, 851-854.
- Bröchner-Mortensen, J. A. (1974). A simple method for the determination of glomerular filtration rate. *Scandinavian Journal of Clinical Laboratory Investigation*, **30**, 271-274.
- Clendinnen, B. G., Reeder, D. D., Brandt, E. N., Jr, and Thompson, J. C. (1973). Effect of nephrectomy on the rate and pattern of the disappearance of exogenous gastrin in dogs. *Gut*, **14**, 462-467.
- Creutzfeldt, W., Arnold, R., Creutzfeldt, C., Feurle, G., and Ketterer, H. (1971). Gastrin and G-cells in the antral mucosa of patients with pernicious anemia, acromegaly and hyperparathyroidism and in a Zollinger-Ellison tumor of the pancreas. *European Journal of Clinical Investigation*, **1**, 461-479.
- Davidson, W. D., Moore, T. C., Shippey, W., and Conovaloff, A. J. (1974). Effect of bilateral nephrectomy and bilateral ureteral ligation on serum gastrin levels in the rat. *Gastroenterology*, **66**, 522-525.
- Durkin, M. G., Essig, L. J., and Nolph, K. D. (1971). Gastrin removal during peritoneal dialysis (Abstract). *Clinical Research*, **19**, 657.
- El Munshid, H. A., Liedberg, G., Rehfeld, J. F., Sundler, F., Larsson, L.-I., and Håkanson, R. (1976). Effect of bilateral nephrectomy on serum gastrin concentration, gastric histamine content, histidine decarboxylase activity, and acid secretion in the rat. *Scandinavian Journal of Gastroenterology*, **11**, 87-91.
- Hansky, J., King, R. W., and Holdsworth, C. D. (1975). Serum gastrin in chronic renal failure. In *Gastrointestinal Hormones*, pp. 115-124. Edited by J. C. Thompson. University of Texas Press: Texas.
- King, R., and Hansky, J. (1974). Serum gastrin after renal transplantation (Letter). *Lancet*, **1**, 169.
- Korman, M. G., Laver, M. C., and Hansky, J. (1972). Hypergastrinaemia in chronic renal failure. *British Medical Journal*, **1**, 209-210.
- Kuku, S. F., Jaspan, J. B., Emmanouel, D. S., Zeidler, A., Katz, A. I., and Rubenstein, A. H. (1976). Heterogeneity of plasma glucagon. *Journal of Clinical Investigation*, **58**, 742-750.

- Lundqvist, G., and Wide, L. (1977). Serum gastrin determination with a radioimmunosorbent technique. *Clinica Chimica Acta*, **79**, 357-362.
- Maack, T. (1975). Renal handling of low molecular weight proteins. *American Journal of Medicine*, **58**, 57-64.
- Maxwell, J. G., Moore, J. G., Dixon, J., and Stevens, L. E. (1971). Gastrin levels in anephric patients. *Surgical Forum*, **22**, 305-306.
- Nakamuro, K., Tanigaki, N., and Pressman, D. (1973). Multiple common properties of human β_2 -microglobulin and the common portion fragment derived from H1-A antigen molecules. *Proceedings of the National Academy of Sciences of the United States of America*, **70**, 2863-2865.
- Newton, W. T., and Jaffe, B. M. (1971). The fate of intravenously administered radiolabeled gastrin. *Surgery*, **69**, 34-40.
- Peterson, P. A., Rask, L., and Lindblom, J. B. (1974). Highly purified papain solubilized H1-A antigens containing β_2 -microglobulin. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 35-39.
- Reeder, D. D., and Thompson, J. C. (1971). Effect of hemodialysis on serum gastrin levels in uremic patients (Abstract). *Gastroenterology*, **60**, 795.
- Rehfeld, J. F. (1972). Three components of gastrin in human serum. *Biochimica et Biophysica Acta*, **285**, 364-372.
- Wibell, L., Evrin, P. E., and Berggård, I. (1973). Serum β_2 -microglobulin in renal disease. *Nephron*, **10**, 320-331.