HUMAN PANCREATIC POLYPEPTIDE AND SOMATOSTATIN IN CHRONIC RENAL FAILURE


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Summary

Human pancreatic polypeptide is the only hormone so far reported which clearly suppresses somatostatin release, suggesting that this peptide may have a role in controlling somatostatin secretion from the gut and pancreas.

In this study endogenous high circulating human pancreatic polypeptide concentrations in patients with chronic renal failure do not decrease somatostatin circulating levels.

The reduced clearance rate of somatostatin in chronic renal failure may partially account for the normal circulating levels of somatostatin observed in our patients with respect to controls.

Renal insufficiency may, itself, induce an increase in some gastrointestinal peptides capable of stimulating somatostatin secretion.

Introduction

The kidneys play a central role in the turnover and regulation of human pancreatic polypeptide [1–3].

Elevated plasma human pancreatic polypeptide concentrations have been found in patients with chronic renal failure [1–4], suggesting the possibility of human pancreatic polypeptide being involved in the development of uraemic gastrointestinal symptoms.

Acute intravenous administration of synthetic human pancreatic polypeptide clearly decreases immunoreactive somatostatin in the rat [5]. It has also been reported that in a patient with a human pancreatic polypeptide cell-tumour who showed extremely high circulating human pancreatic polypeptide levels, plasma somatostatin was undetectable, but rose to a normal level after removal of the tumour [5].

The aim of this study was to evaluate the effect of chronic high endogenous human pancreatic polypeptide plasma levels on circulating somatostatin in man.

The effect of haemodialysis on human pancreatic polypeptide and somatostatin concentrations was also studied.
Materials and methods

Ten patients with stable chronic renal failure (mean creatinine = 2–6 mg/dl) were studied (Group A). After an overnight fast, two baseline blood samples were collected for human pancreatic polypeptide and somatostatin determination.

Twelve age-matched healthy volunteers were examined as controls. In another 12 overnight fasted chronic renal failure patients undergoing chronic dialysis (Group B), blood samples for human pancreatic polypeptide and somatostatin determination were collected before and after haemodialysis.

Results are expressed as mean ± SD. Statistical evaluation was performed using Student’s 't' test.

**Determination of human pancreatic polypeptide**

Immunoreactive human pancreatic polypeptide was measured by a sensitive (4 pg/tube) and validated by radioimmunoassay [6]. After centrifugation, 1 ml of plasma was precipitated with 2 ml of ethanol and supernatant was stored at −20°C until the radioimmunoassay. Highly purified human pancreatic polypeptide (from Dr Ronald Change) was used both for iodination and standard. A standard curve was made in hormone-free plasma and treated as above before use in the assay.

**Iodination** of human pancreatic polypeptide was performed with $^{125}$I using chloramine T as the oxidising agent. The reagents were added in the following sequence and amounts: 100 μl of 100 μg/ml solution of human pancreatic polypeptide (10 μg) in 2 M phosphate buffer pH 7.4, 10 μl of a 100 μCi/ml solution of Na $^{125}$I in 4 M sodium phosphate buffer (1 mCi). The reaction was initiated by adding 10 μl of a 2 g/l solution of chloramine T (20 μg) and was allowed to continue for 15 seconds at room temperature; it was stopped by adding 100 μl of a 5 g/l solution of metabisulphite (50 μg).

**Purification of $^{125}$IPP** the iodinated material was chromatographed on a Sephadex C-25 column (0.9 x 30 cm) and eluted with phosphate buffer 40 mM pH 7.4 with 0.2 per cent human serum albumin at 8 ml/hr speed. Fractions of 1 ml were collected and the radioactivity in each fraction was determined in a gamma counter. The $^{125}$IPP peak arrived between fractions 60–80. Rabbit anti human pancreatic polypeptide serum (lot 204, generous gift from Professor KD Buchanan, University of Belfast) was used at final dilution of 1:48,000.

No crossreactivity was found with other gastrointestinal hormones.

**Conditions for incubation** 0.1 ml ethanol plasma extract and 0.1 ml antiserum were kept at 4°C for 48 hours. Then 0.1 ml $^{125}$IPP (5,000 cpm/tube) was added and the incubation continued at 4°C for a further 48 hours.

Separation of bound from free $^{125}$IPP was performed with 1 ml charcoal-dextran at one per cent (10/1).


**Accuracy and reproducibility** the intra-assay variation of the assay (a measure of accuracy), determined from 25 identical tubes, was 10.2 per cent; the inter-assay variation among 10 assays (a measure of reproducibility) was 16.3 per cent.

**Sensitivity** the smallest amount that can be detected is about 4pg/tube.

**Determination of somatostatin**

Immunoreactive somatostatin was measured by a sensitive and specific radioimmunoassay. After centrifugation 1ml of plasma was precipitated with 2ml of ethanol and supernatant was stored at -20°C until radioimmunoassay. Synthetic somatostatin was used as standard; standard curve was performed in hormone-free plasma and treated as the samples before use in the assay. Iodinated somatostatin was obtained by NEN. Rabbit anti somatostatin (gift from Professor KD Buchanan, University of Belfast) was used.

**Conditions for incubation** 0.1ml ethanol plasma extract and 0.1ml antiserum were maintained at 4°C for 24–48 hours. Then 0.1ml 125I somatostatin was added and the incubation continued at 4°C for a further 48 hours.

Separation of bound from free 125I somatostatin was performed using 1ml charcoal-dextran at one per cent (10/1).

**Accuracy and reproducibility** the intra-assay variation determined from 30 identical tubes was 11.3 per cent; the inter-assay among 15 assays was 14.9 per cent.

**Sensitivity** the smallest amount that can be detected is about 5pg/ml.

**Results**

Basal plasma PP concentrations in Group A (180 ± 50pg/ml) were significantly higher (p<0.001) than those in normal subjects (41 ± 8pg/ml). Mean fasting human pancreatic polypeptide in Group B (725 ± 517pg/ml) did not change after haemodialysis (765 ± 616pg/ml). The degree of human pancreatic polypeptide elevation in patients with chronic renal failure well correlated with the degree of renal insufficiency (p <0.001).

No significant variation in somatostatin was observed between controls: 55 ± 12pg/ml and Group A: 48 ± 18pg/ml.

Group B showed similar basal somatostatin values (52 ± 15pg/ml) which were not significantly modified by haemodialysis (74 ± 22pg/ml).

**Discussion**

Our findings confirm that the mean basal plasma human pancreatic polypeptide is clearly increased in patients with stable chronic renal failure. In the present study a close correlation between the degree of renal insufficiency and plasma
human pancreatic polypeptide increase is further shown. Haemodialysis has no effect on the human pancreatic polypeptide concentration.

Previous studies on the physiological function of human pancreatic polypeptide suggest that somatostatin producing D-cells and human pancreatic polypeptide cells control the secretory activity of each other in a paracrine fashion [5]. It is known that somatostatin exerts an inhibitory effect on human pancreatic polypeptide in man [7]. The suppression of somatostatin by high human pancreatic polypeptide plasma levels has been shown in the rat after acute intravenous injection of the peptide and in a single case of a patient with a human pancreatic polypeptide-cell tumour [5].

In our study mean basal plasma somatostatin value in subjects with chronic renal failure is not reduced with respect to controls, in spite of high circulating levels of human pancreatic polypeptide.

The immediate plasma somatostatin fall observed in the rat following an acute stimulus may be the consequence of a pharmacological treatment and thus the human pancreatic polypeptide inhibitory effect could not necessarily be of physiological significance. The extremely high circulating human pancreatic polypeptide levels in the presence of a human pancreatic polypeptide-cell tumour are not documented as absolute values [5]. Presumably the tumour is able to produce higher plasma human pancreatic polypeptide concentrations than stable chronic renal failure.

In both conditions, however, the renal function was normal. It is known that somatostatin clearance rate is reduced in chronic renal failure, suggesting that the kidney may play a role in the metabolism of the hormone [8,9]. It could partially account for the human pancreatic polypeptide failure to reduce somatostatin levels in the patients studied.

Finally, since various gut hormones and pancreatic glucagon stimulate release of somatostatin [10], stable chronic renal failure could induce an increase of circulating peptides able to maintain a normal plasma somatostatin.

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References

5 Arimura A, Meyers CA, Case WL et al. Biochem Biophys Res Comm 1979; 89: 913
8 Sheppard M, Shapiro B, Pimstone B et al. J Clin Endocrinol Metab 1979; 48: 50