PEPTIDE HORMONES: RENAL HANDLING AND DIALYSIS THERAPY

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Summary

For uraemic diabetics the loss of insulin by dialysis with highly permeable membranes has clinical relevance. We investigated renal handling (in isolated perfused rat kidneys) of insulin and c-peptide and their dialytic clearances and sieving coefficients using cuprophan and polyacrylonitrile membranes. Rat kidney catabolic clearances were five times (insulin) and 12 times (c-peptide) greater than urinary clearances and twice inulin clearance. Cuprophan membrane was found to be virtually impermeable for both hormones. Polyacrylonitrile sieving coefficients were 0.3 (insulin) and 0.4 (c-peptide). Both hormones are filtered as well as tubularly and peritubularly catabolised in the kidney. Insulin removal during dialysis and haemofiltration with polyacrylonitrile membranes has to be considered in treating uraemic diabetics.

Introduction

An increasing proportion of chronic renal failure (CRF) patients with diabetes mellitus are undergoing maintenance dialysis (DIA) therapy [1]. DIA with conventional cuprophan membranes, which are virtually impermeable to solutes with mol wt greater than 5000 daltons has little effect on the removal of larger molecules such as peptide hormones. Following the middle molecule hypothesis [2], which proposed that uraemic toxins may lie in the 300–5000 mol wt range, membranes were developed with greater permeability [3]. The introduction of highly permeable membranes such as polyacrylonitrile (PAN) with increased permeability led to routine use of a new treatment mode, haemofiltration (HF). With the advent of these new membranes and therapies, which facilitate the removal of middle mol wt moieties, the removal of peptide hormones such as insulin and c-peptide need to be evaluated.

The kidney is known to be the major organ involved in catabolism of peptide hormones [4–9]. To assess the renal handling of peptide hormones, urinary clearances and catabolic rates of insulin and c-peptide were investigated using the
isolated perfused rat kidney (IPRK) as a biological model. In addition the removal of insulin and c-peptide during HF and DIA treatments with both cuprophan and PAN membranes were investigated in vitro.

Materials and Methods

The renal handling of insulin (pork insulin, Novo, Copenhagen, 6000 daltons MW) and c-peptide (synthetic, Novo, Copenhagen, 3000 daltons MW) was studied in IPRKs (male HAN-Wistar-rats, 190–330g body weight). Perfusions were conducted in a recirculation system using substrate enriched 50g/l bovine albumin solutions. Volumes of perfusate pools were 144 ± 28ml and 100 ± 5ml (mean ± SD) for insulin and c-peptide respectively. Details of perfusion apparatus and conditions are presented elsewhere [10]. Following an initial period of 30 min perfusion for system equilibration peptide hormone and simultaneous polyfructosan (Inutest, Laevosan, Linz, 4200 daltons MW) clearances of 15–20 min duration were measured during a 60 min experimental period. A control period of 20 min followed each experiment, in which perfusate was recirculated after the kidney was disconnected. Initial pool insulin and c-peptide concentrations were 0.89 ± 0.25 pmol/ml and 2.19 ± 0.23pmol/ml (mean ± SD) respectively. Analysis for insulin and c-peptide were performed using radioimmunoassays [11].

In vitro DIA and HF studies were conducted at 37°C and pH7.4 with both PAN and cuprophan membranes using a plate dialyser with an effective membrane area of 90cm² over four hour periods. Details of dialyser design have been reported previously [12]. Blood solutions consisted of calf sera and the blood-side pool volume and flow rate (Q_B) in all experiments were 32 ± 2ml and 1.0 ml/min respectively. DIA studies were conducted at zero ultrafiltration rates and dialysate flow (Q_D) of 3.0ml/min. In HF studies, transmembrane pressures were 280 ± 10mm Hg for both cuprophan and PAN membranes. Glucose, polyfructosan and peptide hormone clearances (using glucose free dialysate) were determined from pool concentration curves, using 125I insulin and c-peptide.

Results and Discussion

Figure 1 shows IPRK total kidney and urinary clearances for both insulin and c-peptide experiments as functions of time. The simultaneous inulin clearances for the insulin (0.83 ± 0.09) and c-peptide (0.91 ± 0.07ml/min/g kidney — expressed as means ± SEM) experiments are also shown. Over the time course of the experiments total kidney insulin clearances decreased from 2.7 ± 0.7ml/min/g kidney to a steady state of 0.9 ± 0.2, which approached the inulin clearance, while urinary insulin clearances increased from 0.03 ± 0.01 to 0.15 ± 0.06ml/min/g kidney. In c-peptide experiments total kidney clearances rose from initial values of 1.5 ± 0.2ml/min/g kidney to a maximum of 1.9 ± 0.2ml/min/g kidney after 30 min perfusion, while urinary c-peptide clearances increased from 0.02 ± 0.01 to 0.14 ± 0.02ml/min/g kidney during 60 min perfusion. These results indicate that catabolic insulin clearance is five times greater than urinary insulin clearance, while catabolic c-peptide clearance is 12 times greater than urinary c-peptide clearance. Both peptide hormones undergo tubular reabsorption as shown by a comparison
Figure 1. Clearances of insulin and c-peptide at different time intervals in the isolated perfused rat kidney. ○ = total organ clearance, ● = urinary clearance, stippled area = glomerular filtration rate (polyfructosan clearance); mean ± SEM, n = 8

TABLE I.

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<th>DIALYSIS</th>
<th>HAEMOFILTRATION</th>
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<tr>
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<td>Clearances expressed as percentages of glucose clearances</td>
<td>Sieving coefficients</td>
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<td></td>
<td>C-peptide MW 3000</td>
<td>Polyfructosan MW 4200</td>
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<td></td>
<td>Insulin MW 6000</td>
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<tr>
<td>CUP</td>
<td>6 ± 1% (4)*</td>
<td>5 ± 1% (4)</td>
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<td>PAN</td>
<td>19 ± 1% (3)</td>
<td>19 ± 3% (5)</td>
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<td>4 ± 1% (3)</td>
<td>16 ± 1% (3)</td>
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<td><strong>Mean ± SEM (n)</strong></td>
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of GFR and urinary clearance. Since the total clearances for both hormones are greater than GFR, catabolism also occurs at peritubular sites. The time dependent decrease in total insulin clearance may indicate the saturation of insulin binding sites within the kidney. Table I presents sieving coefficients (S) for both hormones.
and polyfructosan with cuprophan and PAN membranes. In the table dialytic clearances are expressed as percentages of simultaneously measured glucose clearances. For cuprophan an increase in solute mol wt is accompanied by a decrease in sieving coefficient. However with PAN, polyfructosan sieving coefficients (0.80 ± 0.01) were about 50% greater than those of the peptides (0.41 ± 0.04 and 0.31 ± 0.04 for c-peptide and insulin respectively). This depression in the sieving coefficient of the negatively charged hormones may be associated with an interaction of the negatively charged protein layer on the surface of the hydrophobic PAN membrane. The non-charged polyfructosan would not experience any charge effects. The dialytic clearances of insulin and c-peptide with cuprophan were less than 6% of glucose clearance indicating that cuprophan is virtually impermeable to the hormones. For PAN dialyses, c-peptide and insulin clearances were less than 20% of glucose clearance. The insulin and c-peptide clearances of a 1m² cuprophan (11.5μ) dialyser at \( Q_B \) 200ml/min and \( Q_D \) 500 ml/min, taking into consideration protein binding, would be 4.3 ± 1.0 and 2.9 ± 0.4ml/min respectively based on the data obtained from our test dialyser. For a 1m² PAN (RP 6) dialyser under similar conditions insulin and c-peptide clearances would be 15.5 ± 0.4 and 18.4 ± 0.5 ml/min.

Conclusions

Normal renal handling, as demonstrated by the isolated perfused rat kidney removes insulin and c-peptide by glomerular filtration and tubular reabsorption, and also by peritubular uptake and catabolism. The removal of insulin and c-peptide from diabetic CRF patients by cuprophan membranes is of no clinical importance [13]. However, although insulin removal by PAN membranes may be low in comparison with normal renal catabolism, it must be taken into account in the management of uraemic diabetics.

References

2. Babb, AL, Farrell, PC, Urelii, DA and Scribner, BH (1972) Trans. ASAIO., 18, 98
5. Rubenstein, AH and Spitz, I (1968) Diabetes, 17, 161
11. Morgan, CR and Lazarow, A (1963) Diabetes, 12, 115
Open Discussion

GODON (Liege) What are the concentrations of insulin in the perfusate of your totally isolated rat kidney? Is it physiological concentration?

SCHLATTER Approximately ten times physiological because we can’t go lower with our radioimmunoassay.

MATTHAEI (Göttingen) As for your last conclusion, we found in haemofiltration the loss of only about one unit of insulin per treatment. That won’t be important for the patient.

SCHLATTER In comparison with cuprophan we found a higher removal rate with the RP 6 but O.K! It is not very big.

MATTHAEI The physiological store of insulin in the islets is very high. One or two units taken off by the dialysis or haemofiltration treatment is nothing, since the islets can produce up to 60 or 70 units of insulin a day.

SCHALATTER Our main interest was the different removal rates between cuprophan and RP 6.

SCHINDHELM (Hannover) The removal rates of insulin with PAN, although impaired by a probable protein layer interaction, should be converted to absolute quantities removed from the individual diabetic patient to assess clinical relevance.

KOPP (München) We have observed, at least in dialysis for acute renal failure with PAN membranes, that glucose tolerance increased and insulin requirement went down during and after dialysis in patients who were dialysed and ultrafiltered up to three litres a day. This is just a clinical observation.

KOKOT (Chairman) Concerning your comment I would like to add that the utilisation of glucose during dialysis of patients with acute renal failure improve significantly, and insulin requirements decrease, i.e. the assimilation coefficient for glucose is improved.