Abnormal Metabolism of Amino Acids in Uraemia

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Impaired glucose tolerance in uraemia has now been well documented. The main underlying pathogenetic mechanism is antagonism to the peripheral action of insulin (Luke et al, 1968; Horton et al, 1968; Spitz et al, 1970). Various abnormalities of plasma amino acid pattern have been described in patients with chronic renal failure both before and after institution of regular dialysis therapy (Gulyassy et al, 1970; Giordano, 1972). In addition negative nitrogen balance and muscle wasting is a common clinical feature of the late uraemic state. Both insulin and growth hormone are necessary for normal metabolism of amino acids (Leatham, 1964; Catt, 1970). The present investigations were performed to study plasma insulin and growth hormone levels following infusion of amino acids, and to determine whether the metabolism of infused alpha amino nitrogen (αAN) is normal as compared to appropriate controls.

PATIENTS AND METHODS

Details of the patients studied are shown in Table I. Five of the uraemic patients had chronic glomerulonephritis, three chronic pyelonephritis with associated urological abnormalities, and one had polycystic kidney disease. All had stable chronic renal failure with no past or family history of diabetes mellitus. They were without oedema, infection or cardiac failure at the time of study and all were clinically in a good state of nutrition. Only one patient in the control and one patient in the uraemic group weighed more than 10% above ideal weight; no patient in either group weighed more than 10% less than ideal weight (Geigy Scientific Tables). No patient had severe metabolic acidosis; mean serum bicarbonate was 24 mEq/l. All controls had a negative urinalysis, normal blood pressure, and a normal creatinine clearance. One patient had received a peritoneal dialysis three weeks before the time of study.
Table I. Clinical features and renal function in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Mean (range)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uraemic</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37 (29-64)</td>
<td>43 (23-65)</td>
</tr>
<tr>
<td>Sex</td>
<td>7M, 2F</td>
<td>8M</td>
</tr>
<tr>
<td>WT (Kg)</td>
<td>70 (46-93)</td>
<td>70 (50-84)</td>
</tr>
<tr>
<td>S. Creat (mg/100 ml)</td>
<td>14.3 (5.4-18.8)</td>
<td>1.3 (1.1-1.5)</td>
</tr>
<tr>
<td>CR. CL. (ml/min)</td>
<td>7 (4-12)</td>
<td>99 (90-111)</td>
</tr>
<tr>
<td>BUN (mg/100 ml)</td>
<td>97 (45-164)</td>
<td>15 (5-24)</td>
</tr>
<tr>
<td>K value (I.V. G.T.T.)</td>
<td>0.92 (6 'Diabetic')</td>
<td>—</td>
</tr>
<tr>
<td>Serum HCO₃ (mEq/l)</td>
<td>24 (15-27)</td>
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All patients ingested at least 200 g carbohydrate daily for 5 days prior to the studies. The uraemic group was being managed by a 40-60 g protein diet. On successive days, after an overnight fast starting at 9:00 pm, patients received, firstly, a standard 25 g intravenous glucose tolerance test (Lundbaek, 1962) at 9:00 am with serial blood specimens, via an indwelling venous cannula, at 0 (fasting), 10, 20, 30, 45, 60 and 120 minutes. Secondly, at 9:00 am on the morning of the second day, 250 ml casein hydrolysate ('Hyprotigen', McGraw) was infused over 40 minutes into a peripheral arm vein. Samples were again withdrawn through an indwelling venous cannula at 0, 20, 40 (end of infusion), 70, 100 and 160 minutes. Plasma αAN, glucose, immunoreactive insulin and growth hormone levels were measured during both the glucose tolerance tests and the casein hydrolysate (CH) infusion. Since normal values for intravenous glucose tolerance test are well established only CH infusions were carried out in controls. In the uraemic patients the order of infusion was varied, so that in four patients amino acid infusion was carried out on the day before glucose infusion and in five the glucose infusion was carried out first. Twenty-four hour urinary collections for estimation of urinary αAN were made on the day of the CH infusion and either the day before or the day after the infusion.

250 ml of CH contained, by analysis, 2,200 mg of αAN and 3,050 mg of total nitrogen (ie 72% of nitrogen was αAN). The amounts of essential amino acids (g) in 250 ml CH, as stated by McGaw, are L-Isoleucine 1.3; L-Leucine 2.1; L-Lysine 1.8; Methionine 0.8; L-Phenylalanine 1.0; L-Threonine 1.0; L-Tryptophan 0.2; L-Valine 1.5 (total essential amino acids 9.5g). In addition the following non-essential amino acids are present: glutamic acid, proline, serine, aspartic acid, alanine, histidine, arginine, glycine, cystine and tyrosine.
From the intravenous glucose tolerance test a constant 'K' was calculated (Lundbaek, 1962)*. Urinary and plasma αAN were measured by the methods of Goodwin (1968 a & b). This method is specific for αAN and is not significantly effected by urea or creatinine. Mean recovery of standards added to uraemic plasma was 103 ± 4% (mean ± SEM) in uraemic plasma and 99 ± 2% from normal plasma. Plasma immunoreactive insulin was measured by the method of Hales and Randle (1962) and immunoreactive growth hormone by the method of Garcia et al (1967) †. Plasma glucose was measured by a glucose oxidase method (Sigma Chemical Bulletin No. 510, a modification of the method of Raabo and Terkildsen (1960)). Plasma creatinine and BUN were measured by autoanalyser.

![Graph showing plasma amino nitrogen levels before, during, and after infusion of casein hydrolysate in uremic patients and control subjects]

**Figure 1.** Plasma αAN before, during and after infusion of casein hydrolysate in uraemic patients and control subjects

* K = 0.693 + T₁/₂, where T₁/₂ is the time in minutes for blood glucose plotted logarithmically to fall by half. In diabetes mellitus K < 0.95, and in non-diabetics K > 1.05, with a mean K of 1.72 in a non-diabetic normal group.

† Standard provided by Endocrinology Study Section, NIAMD, National Institutes of Health, Washington, DC.
Figure 2. Plasma insulin before, during and after infusion of casein hydrolysate in uraemic patients and control subjects

Figure 3. Plasma growth hormone before, during and after infusion of casein hydrolysate in uraemic patients and control subjects
RESULTS

Levels of αAN during and after CH infusion are shown in control and uraemic subjects in Figure 1. Fasting levels were 5.8 ± 0.3 (mean ± SEM) and 6.8 ± 0.5 (NS). Peak levels were observed at the end of the infusion and were 16.0 ± 2.2 (control) and 16.4 ± 1.4 (uraemia). Plasma αAN levels were significantly elevated above control in uraemic subjects at 30, 60 and 120 minutes after infusion.

Urinary αAN measurements were made on 20 occasions in 6 uraemic and 6 control subjects during and either immediately before or immediately after the day of CH infusion. Mean increase in urinary αAN on the day of CH infusion as compared to the control day was 111 mg in control subjects and 46 mg in uraemic patients. This represents 7% of infused αAN in controls and 2% in uraemic subjects. On the day of CH infusion 24 hour αAN excretion was 124 ± 28 mg (mean ± SEM, n=6) as compared to 233 ± 34 mg (n=6) in controls.

Plasma insulin levels during and after CH infusion were higher in uraemics than in controls, significantly so at 30 and 60 minutes after infusion (Figure 2). Plasma growth hormone levels were higher in uraemic than in control subjects, although the differences were not statistically significant (Figure 3).

During glucose infusion in uraemics there was a slight but significant fall in plasma αAN. This fall was usually most marked in the 60 minute value and averaged 0.49 ± 0.08 mg/100 ml as compared to the fasting value (P < 0.001). Changes in plasma glucose during αAN infusion were small. These mean values were (at 0, 20, 40, 70, 100 and 160 minutes) 81, 85, 84, 81, 80 and 83 mg/100 ml in uraemic subjects as compared to 76, 82, 77, 88, 90 and 83 mg/100 ml in controls.

DISCUSSION

We have demonstrated a delay in the rate of disappearance of infused αAN in patients with chronic renal failure, although fasting levels of plasma αAN did not differ between control and uraemic subjects. Others have also found little difference in fasting αAN between normal and uraemic patients (Young & Parsons, 1966; Doyle et al, 1970). The slight increase in urinary αAN losses in the control subjects (7% as compared to 2% of the infused dose of αAN) does not seem adequate to explain the higher levels in the uraemic patients, especially as the peak levels of plasma αAN at the end of infusion were virtually identical in the two groups. Urinary losses normally amount to 1% of the filtered load of amino acids but urinary excretion is maintained despite a progressive drop in glomerular filtration rate, presumably by increased clearance per nephron (Muting & Dishuk, 1967; Gulyassy, 1970; Lathem et al, 1955).
The metabolism of intravenously administered amino acids is largely dependent on peripheral uptake in muscle, whereas, after oral administration, urea formation in the liver is substantial (Munro, 1972; Josephson et al, 1969). Infusion of amino acids is a potent stimulus to insulin secretion (Floyd et al, 1966a) as is oral protein feeding (Floyd et al, 1966b). The stimulus to insulin secretion is produced either by various mixtures of amino acids or by many amino acids given individually. In these experiments αAN reached a level of 13.1 mg/100 ml and peak plasma insulin concentration was 120 μ units/ml, values which are in the same range as those of the present study. Intravenous infusion of amino acids is also a potent stimulus to the secretion of growth hormone (Knopf et al, 1965). Both Floyd et al (1966a, b) and Knopf et al (1965) showed that changes in plasma glucose do not account for secretion of insulin or of growth hormone in response to infused amino acids. The uptake of amino acids into muscle and adipose tissue, and the subsequent synthesis of protein, is strongly increased by growth hormone and insulin in an adjuvant fashion (Leathem, 1964; Catt, 1970). Again these changes are independent of changes in plasma glucose.

In at least one other metabolic disease – diabetes mellitus – there is impaired release of insulin and growth hormone after amino acid infusion (Merimee et al, 1968). The present studies clearly demonstrate that the delay in metabolism of infused αAN in uraemic subjects is not due to diminished circulating levels of insulin or growth hormone. These experiments cannot differentiate between increased secretion of these hormones, and normal or diminished secretion with subsequent diminished clearance in patients with chronic renal failure (Corvilain et al, 1971). Regardless of the mechanism, however, delayed rate of metabolism of amino acids cannot be attributed to insufficient circulating insulin or growth hormone unless immunoreactive measurements do not accurately reflect biologically active hormones.

Delayed rates of metabolism of infused amino acids in the presence of high insulin and growth hormone levels suggest the possibility of antagonism in the uraemic patient to the action of one or both of these hormones. This may be comparable to the insulin antagonism associated with the glucose intolerance of uraemia. In at least one animal model, Lacy (1969) has shown that in acute uraemia there is inhibition of the effect of insulin on the metabolism of amino acids.

The phenomenon of delayed metabolism of amino acids in uraemic patients merits further dissection and study.
REFERENCES

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OPEN DISCUSSION

DR R KLUTHE (Chairman): Thank you very much. Dr Luke's paper is now open for discussion.

F M PARSONS (Leeds): We are very interested in this topic. How much part do you think is being played by abnormalities of liver function, not measured by crude liver function tests? I think that anybody that has looked at enzymic action in amino acid metabolism has found a marked decrease in enzymic activity. Could this be what you are estimating?

LUKE: The literature would suggest that when one infuses intravenous amino acid as done here, perhaps 70 or 80 per cent of the metabolic disappearance is due to the passage of amino acids into muscle and fatty tissue. Certainly when one takes an oral amino acid, the liver plays a much greater part but it is probably not important here. We have not done any studies of the rates of disposal but I think that there is probably diminished passage of amino acids over the muscle membranes, or a subsequent block within the cell.

C GIORDANO (Naples): By increasing the caloric intake per kg for your patients up to 55 cals/kg of body weight, would you get the same kind of results. If this were true, then you could substantiate your hypothesis of a membrane being affected by the insulin antagonist.

LUKE: We do not have the data to answer your question. All of the patients were stable and according to our dietitian were eating an adequate diet, but I think that serial studies of this nature in the same patient before and after transplantation, and on regular dialysis therapy might be very interesting.