THE EFFECTS OF LOW-PROTEIN DIET AND URAEMIA UPON UREA-CYCLE ENZYMES AND TRANSAMINASES IN RATS

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SUMMARY

1. Activities of arginine synthetase system enzymes, alanine aminotransferase (AlT) and branched-chain amino acid transaminase (BATase) were measured in control and uraemic rats after 2 weeks on a free protein intake and after 10 days on a low-protein diet.

2. Protein restriction alone reduced the activity of all three enzymes.

3. Uraemia alone increased the activity of arginine synthetase system enzymes and AlT. BATase was not altered.

4. After protein restriction the BATase activity in uraemic rats decreased to the same extent as in controls. The activity of arginine synthetase system enzymes and AlT were significantly higher than in protein-restricted controls and were the same as in control rats on a normal diet.

5. Practical consequences of the finding that uraemia prevented the decrease of AlT but not of BATase activity in response to a low-protein diet are discussed, with particular reference to reutilization of urea-nitrogen for the synthesis of non-essential and essential amino acids.

Key words: urea-cycle enzymes, transaminases, low-protein diet, uraemia.

Recycled urea-nitrogen is used in uraemia for the synthesis of non-essential amino acids (Giordano, 1963; Richards, Metcalfe-Gibson, Ward, Wrong & Houghton, 1967; Giordano, De Pascale, Balestrieri, Cittadini & Crescenzi, 1968). This nitrogen is also available for the synthesis of some essential amino acids if their carbon skeletons are provided in the diet (Richards, Brown, Houghton & Thompson, 1971; Giordano, Phillips, De Pascale, De Santo, Fürst, Brown, Houghton & Richards, 1972). The most uraemic man we have studied in this respect synthesized both phenylalanine and valine (Giordano et al., 1972) but failed to maintain nitrogen balance (Richards et al., 1971). Decreased transaminating capacity might have been responsible for his relatively inefficient transamination of the α-ketoacid analogues. We
report here the effect of protein restriction and uraemia in rats on the activity of L-alanine-2-oxoglutarate aminotransferase (EC 2.6.1.2., alanine aminotransferase: ALT) and arginine synthetase system enzymes in liver, and of branched-chain amino acid transaminase (BATase) in muscle, the tissue in which it is most abundant.

MATERIALS AND METHODS

Outbred male Wistar rats, initial weight 245–323 g, were divided into control and uraemic groups. The latter group was made uraemic by right partial nephrectomy under ether anaesthesia; both poles of the kidney were excised together with some of the cortex of the remaining cylinder of kidney; the cut surfaces were cauterized. A left nephrectomy was performed 4 or 5 days later. Some control animals had a left partial nephrectomy but no right nephrectomy; others had no operation. No difference was found between the operated and unoperated controls and they are considered together.

For the first 2 weeks all rats were fed ad libitum upon a stock diet which contained 33 mg of N/g as determined by Kjeldahl digestion and distillation. During this period the subtotally nephrectomized rats developed stable uraemia, with a mean blood urea of 268 mg/100 ml ± 22 SEM. One control and one uraemic rat was then killed each day for 5 days by a sharp blow on the head. Animals were killed at the same time each morning but were not starved before. The remaining rats were then fed ad libitum upon the stock diet diluted with dextrin and corn oil (200 g of pellets : 800 g of dextrin : 100 ml of oil) to give a concentration of 6 mg of N/g. Equal numbers of control and uraemic rats taking this low-protein diet were killed on each of the 10th–14th days. On the low-protein diet the blood urea concentration of uraemic rats fell to 67 mg/100 ml ± 10 SEM.

The livers and part of the thigh muscle were excised and washed in ice-cold 0·05 M-potassium phosphate buffer, pH 7·4. Arginine synthetase system enzymes were assayed by the method of Brown & Cohen (1959), except that the reaction was stopped by the addition of 1·0 ml of 1·0 M-perchloric acid. The precipitated protein was removed by centrifugation and the supernatant was adjusted to approximately pH 7 with NaOH. Urea was then estimated by the urea phenate method (Chaney & Marbach, 1962). Homogenates for transaminase determinations were prepared in ice-cold 0·05 M-potassium phosphate buffer, pH 7·4, and spun at 3000 g for 15 min at 0°C. ALT activity was measured in the liver supernatant by using u.v. system glutamic pyruvate transaminase activity tests (Boehringer G.m.b.H., Mannheim, Germany). BATase activity was measured in the muscle supernatant using α-ketoisovalerate as substrate by the method of Ichihara & Koyama (1966). Samples were incubated for 60 min; a control incubation was carried out for each tissue sample without addition of substrate to estimate endogenous ketoglutarate. This control value was subtracted from the apparent BATase activity. The activity of BATase in liver for the conversion of α-ketoisovalerate into valine was too low for consistent measurement. Its activity upon the conversion of valine into α-ketoisovalerate was greater and was measurable but we were particularly concerned with the transamination of keto acid to amino acid. Consequently we measured BATase activity in muscle which showed higher activity in this direction. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine albumin as standard. The difference between the mean enzyme activities was analysed by Student's t-test.
RESULTS

The mean blood urea of uraemic animals on normal and low-protein diet was 268 mg and 67 mg/100 ml respectively. The corresponding values in controls were 40 mg and 24 mg/100 ml (Table 1). At the time animals were killed all groups weighed less than controls on normal diet and their liver mass was reduced (Table 1).

### Table 1. Body weights, liver weight and blood urea at time of death

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Blood urea (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Uraemic</td>
<td>Control</td>
</tr>
<tr>
<td>Normal</td>
<td>340 ± 10.4</td>
<td>251 ± 11.4</td>
<td>13.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(12)</td>
<td>(10)</td>
</tr>
<tr>
<td>Low-protein</td>
<td>289 ± 10.5</td>
<td>246 ± 8.6</td>
<td>9.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(10)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM with the number of observations in parentheses.

### Table 2. Tissue enzyme activities

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>n</th>
<th>Arginine synthetase system (nmol of urea produced mg of protein⁻¹ h⁻¹ ± SEM)</th>
<th>Alanine aminotransferase (nmol alanine converted mg of protein⁻¹ h⁻¹ ± SEM)</th>
<th>Branched-chain amino-acid transaminase (nmol α-ketoglutarate converted mg of protein⁻¹ h⁻¹ ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Normal</td>
<td>10</td>
<td>735 ± 46</td>
<td>5380 ± 530</td>
<td>60.6 ± 5.9</td>
</tr>
<tr>
<td>Controls</td>
<td>Low-protein</td>
<td>9</td>
<td>488 ± 42**</td>
<td>3680 ± 260**</td>
<td>42.3 ± 3.6**</td>
</tr>
<tr>
<td>Controls</td>
<td>Normal</td>
<td>10</td>
<td>735 ± 46</td>
<td>5380 ± 530</td>
<td>60.6 ± 5.9</td>
</tr>
<tr>
<td>Uraemics</td>
<td>Normal</td>
<td>12</td>
<td>916 ± 64**</td>
<td>8390 ± 730**</td>
<td>55.6 ± 5.4</td>
</tr>
<tr>
<td>Controls</td>
<td>Low-protein</td>
<td>9</td>
<td>488 ± 42</td>
<td>3680 ± 260</td>
<td>42.3 ± 3.6</td>
</tr>
<tr>
<td>Uraemics</td>
<td>Low-protein</td>
<td>10</td>
<td>659 ± 32**</td>
<td>5570 ± 440**</td>
<td>45.3 ± 4.3</td>
</tr>
<tr>
<td>Controls</td>
<td>Normal</td>
<td>10</td>
<td>735 ± 46</td>
<td>5380 ± 530</td>
<td>60.6 ± 5.9</td>
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</tr>
</tbody>
</table>

Significance of difference relates to each pair of comparisons: *P < 0.05, **P < 0.001.

Enzyme activities have been expressed per mg of tissue protein, although the significance of the differences observed was the same whether the activities were expressed per mg of tissue protein or per g of wet tissue.

Protein restriction alone decreased the activity of all three enzymes (Table 2). Uraemia per se increased both arginine synthetase and AIT activities but did not significantly alter BATase activity when the diet was normal. Uraemic animals on a low-protein diet had significantly higher activities of arginine synthetase and AIT than protein restricted controls; their BATase activity was the same as the controls. Although protein restriction of uraemic animals caused decrease in all three enzyme activities only BATase activity fell below the activity found in
control rats on a normal diet. In all circumstances the BATase activity was only about 1% of A1T activity.

DISCUSSION

Knowledge of changes in enzyme activity during adaption to changes in protein intake is fragmentary. As far as we are aware, transaminase and urea-cycle enzyme changes in response to uraemia alone and to uraemia and protein restriction together have not previously been investigated.

Our experiments have confirmed the observations of Schimke (1962) in rats and Stephen & Waterlow (1968) in infants that protein restriction decreases the activity of urea-cycle enzymes. Likewise we found lower A1T activity. It is difficult to compare the fall in BATase in muscle during protein restriction with the findings of others. Mimura, Yamada & Swendseid (1968) found an increase in BATase activity in liver, muscle and intestine in response both to a high-protein diet and to a protein-free diet. In kidney, the tissue which contained a higher concentration of BATase than any other tissue, the concentration increased in response to a high-protein diet but fell significantly on a protein-free diet. Mimura et al. (1968) found higher concentrations of BATase than either Ichihara & Koyama (1966) or us, a difference which may be partly explained by the use of different strains of rat. Our values would be expected to be lower because the activity of BATase in the conversion of keto acid into amino acid is only about half of the activity measured by the conversion of amino acid into keto acid (Ichihara &

Fig. 1. Regression of arginine synthetase system activity on blood urea: \( y = 1.21x + 577, \ r = 0.65, \ P < 0.001 \). \( \triangle \), Uraemic normal diet; \( \Delta \), uraemic low-protein diet; \( \bigcirc \), controls on normal diet; \( \bullet \), controls on low-protein diet.
Transaminases in uraemia

Koyama 1966), the direction in which Mimura et al. (1968) measured it. In common with these authors we allowed a period of 10 days for enzyme changes to stabilize.

In uraemia, arginine synthetase system enzyme activities (of which argininosuccinate synthetase is the rate-limiting enzyme in the Krebs–Henseleit urea cycle) were increased in direct proportion to the blood-urea concentration \((r=0.649, \ P<0.001, \text{Fig. 1})\). This finding is a predictable result of the substantial increase in ammonia which reaches the liver in uraemia from urea hydrolysis in the colon (Walser & Bodenlos, 1959). It is not known whether the decrease in urea-cycle enzyme activities found during protein restriction results in diversion of recycled ammonia to glutamic acid synthesis or whether it is only a reflection of the quantity of ammonia reaching the liver in portal blood.

![Fig. 2. Regression of AIT activity on blood urea: \(y=15x+4260, \ r=0.67, \ P<0.001\). Symbols are the same as in Fig. 1.](image)

AIT activity also increased in proportion to the blood urea \((r=0.672, \ P<0.001, \text{Fig. 2})\). Because AIT activity was very significantly increased by uraemia and decreased by protein restriction we noted the effect of combined uraemia and protein restriction. Re-utilization of ammonia nitrogen depends in the first place upon the fixation of ammonia by reductive amination of \(\alpha\)-ketoglutaric acid to glutamic acid, then upon the transamination of other \(\alpha\)-keto acids with this nitrogen. AIT activity per mg of protein in the liver of protein-restricted uraemic animals proved to be greater than in protein-restricted controls and as great as in animals on a normal diet. Although we have no direct evidence concerning the nature of the stimulus which caused increased AIT activity it may have resulted from increased amounts of ammonia reaching the liver from urea hydrolysed in the colon.

The observation that in protein-restricted uraemic animals AIT activity was particularly well preserved relative to BATase activity would, if true also of man, provide one possible
reason why synthesis of phenylalanine by uraemic patients might be more efficient than the synthesis of valine (Richards et al., 1971). Synthesis of essential amino acids may be a special case, for there is no evidence of impaired re-utilization of non-protein nitrogen in protein-restricted individuals; in fact the reverse is true. Wastage of amino acids from catabolized protein is decreased (Waterlow, 1968) and incorporation of ammonia nitrogen into albumin increases (Richards et al., 1967).

ACKNOWLEDGMENTS

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REFERENCES


