CEREBRAL AND HEPATIC UREA SYNTHESIS IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY

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Summary

Urea production by the liver and the brain was evaluated in patients with chronic renal insufficiency and in subjects with normal renal function by measuring the arterial-venous differences of urea across the hepatosplanchnic bed and the brain. In five out of seven patients with chronic renal insufficiency no urea release into the hepatic veins was observed, whereas a high urea output by the brain was measured in 6 out of 8 patients. In the control group urea was released only into the hepatic veins. These data demonstrate a defect in hepatic urea synthesis and a switch to cerebral ureagenesis in chronic renal insufficiency.

Introduction

It is well established that chronic renal insufficiency (CRI) induces severe alterations in nitrogen metabolism and enzymatic activity [1]. Nevertheless, the overall urea balance, studied by isotope methods, does not show striking differences between normal subjects and patients with CRI [2,3]. In those studies urea synthesis is assumed to occur only in the liver, which in normal conditions is believed to be the exclusive site for urea production through the ornithine cycle [4]. However the isotope methods, employed for overall balance studies, cannot provide direct information on the site of urea production. Accordingly, even in the presence of an almost normal overall urea balance in CRI, possible alterations in the hepatic urea cycle and even extrahepatic urea production cannot be ruled out. If extrahepatic urea production is suspected, the brain is one of the major organs to be investigated. Although the brain does not contain the ornithine cycle, it is provided with pathways for urea production from citrulline, argininosuccinate and arginine [5–7]. Researches here reported were performed in order to evaluate hepatic urea production in CRI and at the same time search for possible cerebral urea synthesis.
Materials and Methods

Studies were carried out on patients admitted to the Department of Internal Medicine of the University of Genoa between 1974 and 1978. The patients were informed of the purpose, nature and procedure involved in this research which was performed only after the patients gave their consent.

Patients with CRI

Nine patients of both sexes, between the ages of 32 and 58, with CRI and GFR (C-thiosulphate) from 10 to 26 ml/min were studied. All patients had been on a diet providing from 35 to 45 g of protein daily for 4 months at least. Their body weight was steady. There was no history or evidence of congestive heart failure, pulmonary or hepatic disease or diabetes. A mild or moderate anaemia was present. Blood urea was between 11.8 and 38.5 mmol/L and serum creatinine was between 0.193 and 0.721 mmol/L. Serum sodium and potassium levels were normal and urine cultures were repeatedly negative. Eight patients had suspected hyperparathyroidism and one was moderately hypertensive, therefore an internal jugular vein catheterisation for parathormone assay or a renal vein catheterisation for plasma renin activity determination was considered necessary as part of their diagnostic evaluation.

Patients with Normal Renal Function

Eleven patients of both sexes, between the ages of 28 and 55, were studied. Four patients had arterial hypertension and 7 had cardiac valvular diseases. Routine haematological tests, serum and urine electrolytes, acid-base parameters, urinalysis and renal function tests were normal ($C_{\text{creatinine}} = 102-128$ ml/min/1.73m²). All patients were on a normal diet. In hypertensive patients a renal vein catheterisation had to be carried out in order to determine plasma renin activity. The other patients had to undergo a right sided cardiac catheterisation for haemodynamic evaluation.

Measurement of Arterial-venous Differences

All patients were studied after a 12–14 hr overnight fast. A teflon catheter was placed percutaneously into a peripheral artery in order to measure acid-base parameters and arterial urea concentration. Then a catheter (Courmand No.6 or 7 F or ‘Cobra’ No.6 or 7 S) was guided, under fluoroscopic control, through either an antecubital vein or a femoral vein, to a hepatic vein or to the superior bulb of an internal jugular vein. From each subject at least two sets of simultaneous arterial and venous samples were obtained for the measurement of arterial-venous differences of urea concentration across the organs. Arterial blood pressure and electrocardiogram were continuously monitored during the study. Blood, withdrawn by heparinised syringes kept in ice, was used for pH and pCO₂ measurements. An aliquot of the same sample was rapidly centrifuged at +4°C. Plasma was routinely used for the determination of urea concentration. During
catheterisation urine was collected for a 2 hour period under mineral oil in bottles containing thymol.

**Analysis**

Urea was determined enzymatically by the Chaney and Marbach method [8]. Plasma proteins were precipitated with 0.75 M perchloric acid and the supernatant neutralised with a buffered solution. An aliquot of the supernatant was incubated at 37°C and pH 6.5 with 0.6 U/test tube of urease (Grade VII, Sigma Chemical Corp., St Louis, Mo). After a 15 min incubation the ammonia released was measured by the Chaney and Marbach method [8]. Each sample containing the enzyme was compared with a sample without the enzyme and with a set of urea standards. An enzyme blank was also used. Samples and standards for each assay were all in triplicate; each assay was repeated 4–6 times. Recovery of urea added to plasma was determined for each assay: recovery ranged from 97 to 102%. Statistical significance of arterial-venous urea concentration differences was examined for each patient by analysis of variance using a randomised block design. Urea in urine was also determined enzymatically [8].

Sodium thiosulphate concentration in plasma and urine for GFR measurement was determined according to Brun [9]. Creatinine in plasma and urine was determined after absorption on to Lloyd's reagent [10]. Blood pH and pCO₂ were estimated at 37°C with a PHM 72/BMS3 Apparatus (Radiometer, Copenhagen). Blood [HCO₃⁻] was calculated using the Henderson-Hasselbalch equation.

Statistical significance was examined by analysis of variance using a completely randomised design or a randomised block design [11]. Values are given as mean ± 1 SEM.

**Results**

In 5 out of 7 patients with CRI no statistically significant urea release into the hepatic veins was detected. The mean arterial-venous difference in the group as a whole was −33 ± 12.7 µmol/L. On the contrary, a high urea release into the hepatic veins (A-V = −177 ± 29.4 µmol/L) was measured in all subjects with normally functioning kidneys (Table I). In 7 subjects with normal renal function no statistically significant difference in urea concentration between arterial and internal jugular veins was observed (A-V = +59 ± 24 µmol/L), whereas a high output of urea by the brain was measured in 6 out of 8 patients with CRI. The mean arterial-venous difference in the group as a whole was −225 ± 49.7 µmol/L (Table I). During catheterisation procedures urea excreted in the urine was 302 ± 39.2 µmol/min in the 11 subjects with normal renal function, and 216 ± 14.4 µmol/min in the 9 patients with CRI (Table I).

**Discussion**

It is well known that in normal conditions the liver is the exclusive site for urea production [4] and the intestines degrade it significantly [12–14], decreasing the urea concentration in portal blood. Since the relative blood flow
TABLE I. Hepato-splanchnic and Cerebral Metabolism of Urea in Patients with Chronic Renal Insufficiency and in Subjects with Normal Renal Function

<table>
<thead>
<tr>
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<th>GFR ml/min/1.73 m²</th>
<th>[Urea]ₐ µmol/L</th>
<th>Urinary urea µmol/min</th>
<th>Arterio-hepatic venous differences µmol/L</th>
<th>Arterio-internal jugular venous differences µmol/L</th>
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<tr>
<td>Patients with chronic renal insufficiency</td>
<td>19.4 ± 2.19 (9)*</td>
<td>20.1 ± 2.71 (9)*</td>
<td>216 ± 14.4 (9)†</td>
<td>-33 ± 12.7 (7)*</td>
<td>-225 ± 49.7 (8)*</td>
</tr>
<tr>
<td>Subjects with normal renal function</td>
<td>121.6 ± 8.08 (11)</td>
<td>4.6 ± 0.40 (11)</td>
<td>302 ± 39.2 (11)</td>
<td>-177 ± 29.4 (8)</td>
<td>+59 ± 24.0 (7)</td>
</tr>
</tbody>
</table>

[Urea]ₐ = arterial urea concentration
* p < 0.01 compared with subjects with normal renal function
† p < 0.05 compared with subjects with normal renal function
Results are expressed as mean ± 1 SEM

through the liver derives approximately 75% from the portal vein and 25% from the hepatic artery, urea concentration in hepatic veins reflects a balance between hepatic synthesis and intestinal degradation of urea. Therefore intestinal degradation can mask hepatic synthesis to different extents, so that a decreased concentration in hepatic veins may be independent of variations in hepatic synthesis. Our study cannot establish whether the lack of a significant urea release into the hepatic veins observed in most patients with CRI reflects an increase in intestinal breakdown and/or a decrease in hepatic production. However intestinal urea breakdown does not differ substantially in normal conditions and in CRI [2,3]. Accordingly, our results strongly suggest a defect in hepatic urea synthesis occurring in most patients with CRI. It is unlikely that such defect depends on the diet, since the daily protein intake of the patients here reported was only moderately restricted. Moreover their urea excretion in urine was fairly high, and consistent with their protein uptake. Nor is the 14—16 hour fasting condition, under which the patients were studied, responsible for defective hepatic urea production, since the same procedure was followed in subjects with normal renal function, who conversely showed high urea outputs into the hepatic veins.

In conclusion, our data strongly suggest extrahepatic urea production in CRI. Such a possibility is confirmed by the net release of urea into the jugular veins, observed in most patients with CRI. It is not possible to express arterial-internal jugular venous differences of urea as production rates, since we could not measure the blood flow across the brain. Nevertheless, our results demonstrate a surprising cerebral ureagenesis appearing with the disappearance of the normal urea release into the hepatic veins. There are no data in the literature comparable with our results. However urea production by the brain was shown in dogs treated with pentobarbital [15]. This substance also depresses hepatic urea synthesis in the same animal [16].

So far we cannot explain the defect in hepatic urea synthesis and the activation
of cerebral ureagenesis in CRI, but several hypotheses must be considered. The
defect in hepatic urea synthesis might be caused by some enzymatic inhibition
within the urea cycle. In fact a decrease in ornithine-carbamyltransferase, but
not in arginosuccinate synthetase and arginosuccinase activities, was demon-
strated in the liver of uraemic rats [17]. Other enzymatic alterations before the
ornithine-carbamyl transferase step may be involved. Moreover alterations in
 glutamine metabolism, besides that of other aminoacids, must be taken into
account. It is known that glutamine is an important precursor of urea, providing
20% of urea nitrogen excreted by the rat [18]. There is also evidence that glut-
amine metabolism across the hepato-splanchnic bed is altered in patients with
CRI, where the normal hepato-splanchnic uptake of glutamine disappears [19,20].
Such alteration could limit the availability of N for hepatic urea synthesis. The
brain does not contain the ornithine cycle, in fact it cannot produce carbamyl-
phosphate nor citrulline, but it can produce urea from citrulline, arginosuccin-
ate and arginine [5—7]. Accordingly, specific alterations acting above citrulline
could not hinder urea production from citrulline nor from substrates below it.
Even if the hepatic urea production is impaired in CRI, the liver nevertheless
maintains an important role in such production by supplying the brain with sub-
strates, including perhaps citrulline, for urea synthesis.

In conclusion, our data indicate that in most patients with CRI hepatic urea
synthesis is defective and the brain seems to assume an important role in urea
production.

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Open Discussion

ÖNEN (Istanbul) I have three questions. Did you also measure the amino acid uptake by the liver, by determining the amino acid concentration in hepatic artery and in hepatic veins? Secondly, do you know anything about splanchnic blood flow in these cases, especially in uraemics? Thirdly, is there any possibility that in uraemic patients there is a decrease of the hepatic amino acid uptake as a consequence of a decreased amino acid reabsorption by the gut?

TIZIANELLO We are now measuring arterial-hepatic venous differences of amino acid concentrations in patients with chronic renal insufficiency. So far we have no definite data. We suspect that an increased citrulline release into the hepatic veins might be found. We suppose that citrulline could be utilised by the brain as a substrate for urea production. Blood flow measurements would have further complicated the procedure followed for these studies. Consequently, we cannot explain our data as rates of production. However, the difference between patients with renal insufficiency and controls are striking and the phenomenon are clearly outlined. I cannot answer your third question. Anyhow our patients were studied in post-absorptive state.

VLAHO (Cologne) We found that urea synthesis is diminished in the livers of chronically uraemic rats. Have you any explanation for this? Is the energy supply diminished in chronic uraemia?

TIZIANELLO We have no date about energy supply or urea cycle enzymes in chronic renal insufficiency. As I said, the ornithine-carbamyl-transferase is significantly inhibited in uraemic rats. We found that glutamine metabolism across the hepato-splanchnic bed is altered in chronic renal insufficiency when the normal uptake disappears (Tizianello et al (1978) Clin. Sci. Mol. Med., in press). There is good evidence that glutamine is one of the major precursors of ammonia for the carbamyl phosphate synthesis. Therefore it is possible that in chronic renal insufficiency glutamine does not provide ammonia for urea synthesis in the liver.

BUCHT I do not understand why you could not measure both liver and cerebral blood flow - it is a simple technique. Your results should be expressed as urea production rates for brain and liver.

TIZIANELLO I agree with you: it is not difficult to measure the hepato-splanchnic and cerebral blood flows. We can measure these blood flows by cardioremark. I want to stress that blood flow measurement would further complicate the procedure, for which it is necessary to have the patient’s consent.