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Biliary Amino Acid Excretion in Rats before and after Bilateral Nephrectomy

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Summary

In previous studies it could be shown that after bilateral nephrectomy (NX) the excretory function of the liver is disturbed. To further clarify whether or not this "renohepatic syndrome" is caused by toxic effects of uremia or by competition phenomena between variuos uraemic toxins an additional aspect was investigated: the biliary excretion of endogenous amino acids. Furthermore, previously it could be shown that renal and hepatic excretory functions overlap. Therefore, the renal excretion of effectively biliary eliminated amino acids (glutamic acid, alanine, tyrosine, isoleucine) is very low and *vice versa*. That means, that the renal excretion of amino acids with low hepatic elimination (tryptophan, citrulline, lysine, taurine) dominates. The hepatic excretion of amino acids is hardly altered after NX. Remarkably, the removal of both kidneys is followed by a distinct reduction in amino acid plasma concentrations, especially if these concentrations are relatively high in the controls. Interestingly, there is no correlation between plasma concentrations and biliary excretion of amino acids. But the calculation of the bile to plasma concentration ratios of amino acids makes it possible to differentiate three groups of amino acids: Amino acids excreted actively into bile (ratio ≥ 1), amino acids with ratios below 1, indicating effective retention, and amino acids with ratios of about 1, whose hepatic handling is passive. After NX these ratios tended to approach 1; low ratios increased and high ratios decreased. That means, active processes involved in excretion or retention are obviously disturbed. These changes could indicate uraemic liver damage as proved regarding influence of NX on hepatic excretion of other endogenous substances and xenobiotics.

Key words

Biliary excretion – Hepatic transport – Amino acid excretion – Uremia – Nephrectomy

Introduction

Amino acids play an important role in the functioning of mammalian tissues, serving as metabolic substrates in the small intestine, kidney, liver, and as excitatory transmitters in the central nervous system (Berteloot and Maenz 1990). It is well established that urinary excretion of amino acids is normally very small if compared with the filtered load (Silbernagl 1988). But little is known concerning the biliary excretion of amino acids. Without doubt this pathway is of minor importance for amino acid balance of the organism, because effective reabsorption mechanisms exist in the gastrointestinal tract which prevent the biliary loss of amino acids (Hopfer 1986). The hepatic uptake of amino acids is a carrier-mediated process (Leoni *et al.* 1988). Eight main routes serve for amino acid transport within the liver (Kilberg 1982). The only amino acids whose mechanism of biliary excretion is known are glutamate, cyst(e)ine, and glycine, the three amino acid constituents of glutathione (Ballatori *et al.* 1986a,b). However, in the literature there is no information about what happens if the liver is diseased. The biliary loss of amino acids could be important, if membranes of the hepatocytes become permeable to amino acids.

Previously it has been shown that uremia caused by bilateral nephrectomy (NX) performed 24 hours before an experiment significantly impaired liver function (Fleck *et al.* in press). Both toxic effects of so called uraemic toxins (Ringoir *et al.* 1988) and competition phenomena between endogenous substances, whose plasma concentrations are enhanced after kidney removal, could influence the hepatic transport capacity. A specific aspect of hepatic transport consists in the handling of amino acids within the liver. Changes of the concentration of amino acids in blood and bile after NX could indicate either toxic hepatic damage followed by disturbances in amino acid metabolism in the liver (Fürst 1989) or competitive mechanisms concerning amino acid uptake and

transport within the hepatocytes (Bucuvalas *et al.* 1986). Therefore, in this study the concentrations of endogenous amino acids were determined in the blood and bile of rats before and after NX.

Material and methods

Preparation of animals for the experiments:

Male Wistar rats (Ivanovas, Kisslegg, Germany) weighing 160-200 g were fed on an Altromin standard diet and had free access to tap water. The rats were anaesthetized with 12 mg/100 g b.wt. thiobutabarbital (Inactin®, Byk-Gulden, Konstanz, Germany) intraperitoneally. A tracheostomy was performed and a polyethylene tube was placed in the left jugular vein. The animals were then given an infusion of Ringer's solution at a priming rate of 4.5 ml/100 g b.wt. per hour for 15 min and then at 2.25 ml/100 g b.wt. per hour for the remainder of the experiment. In nephrectomized rats, the infusion volume was reduced by 50 % to replace the biliary fluid loss and to keep the blood pressure constant. The Ringer solution had the following composition (in g/l): 9 NaCl, 0.4 KCl, 0.25 $CaCl_2$ and 0.2 $NaHCO_3$. The abdominal cavity was then opened, and the common bile duct was dissected and a polyethylene tube inserted into its proximal third. The urinary bladder was catheterized. Bile and urine were collected for one hour in three 20-min collecting periods. In the middle of each clearance period, blood was obtained from the retrobulbar plexus; at the end of the experiment the rats were decapitated and blood was collected.

In further experiments, the effect of bilateral nephrectomy (NX) on the hepatic excretion of amino acids was tested 24 hours before the clearance study when both kidneys were removed under hexobarbital anaesthesia (Hexobarbital Natrium®, AWD Dresden, Germany, 10 mg/100 g b.wt. intraperitoneally). The abdominal cavity was opened by a flank side incision, the kidneys were decapsulated, the hili ligated, and the kidneys removed. The rats woke up 30 to 60 min after the end of the surgical intervention.

Amino acid determination

The determination of amino acids by column chromatography with fluorescence detection is based on that developed by Roth and Hampai (1973) and has been described in detail elsewhere (Silbernagl 1983). Briefly, proteins were removed from bile, urine, and plasma samples by administration of trichloroacetic acid (10 %). After centrifugation the supernatant was made neutral by adding of 0.4 N NaOH. Then the samples were diluted with Li^+-citrate buffer (pH 2.70, 0.2 mol Li^+/l) and analyzed by column chromatography on a Chromakon 500 analyzer (Fa. Kontron, München) with o-phthalaldehyde as a fluorescing amino ligand (Roth 1971). Calibration runs were performed with freshly prepared amino acid solutions made with analytical grade amino acids (Serva, Heidelberg, Germany).

Statistical analysis

Results are summarized as means ± S.E.M.; n=6 in all groups. The level of significance for differences between observations was assessed with Student's test. Differences determined by t-test were considered statistically significant when $p \leq 0.05$.

Results

The transport of amino acids in the kidney and the liver is intended to preserve these essential substances from excretion. Therefore, net excretion of amino acids is relatively low. The total excretion amounts to about 2.28 and 3.67 μmol/100 g b. wt. per 1 hour *via* urine and bile, respectively. There is no correlation between plasma concentrations of amino acids and the degree of their renal or hepatic excretion. However, there is a correlation between the ratio of the renal to the hepatic amino acid excretion and the hepatic elimination of amino acids (Fig. 1).

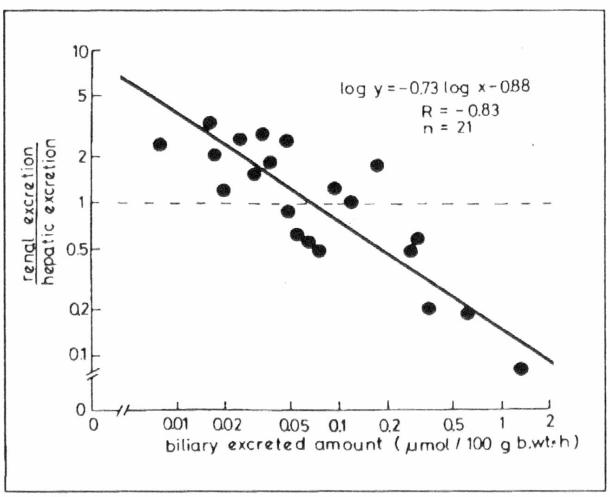

Fig. 1

Correlation between the amounts of various amino acids excreted *via* the bile and the ratio between their renal and hepatic excretion route. Each point represents one of the amino acids. n = 6 for each amino acid.

This means that for amino acids excreted effectively into the bile (for absolute values cf. Fig. 2), the renal excretion is relatively low compared with their hepatic removal and *vice versa*. From this point of view the question arose as to what happens after blockade of the

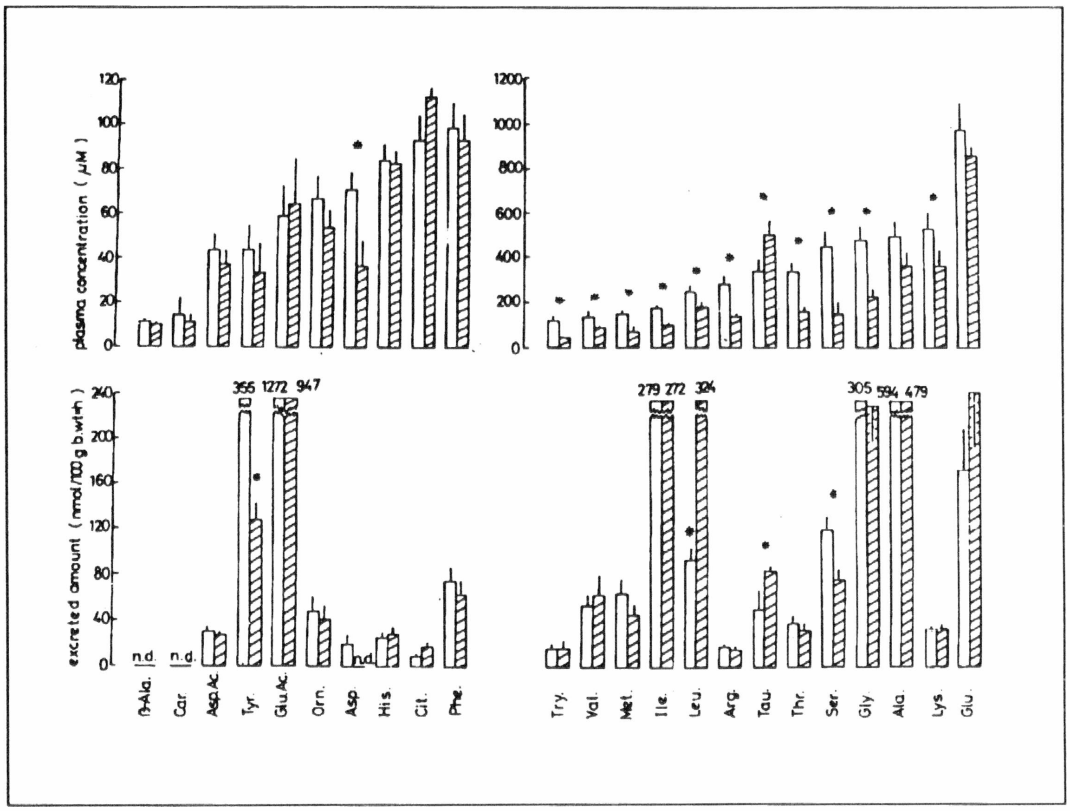

Fig. 2

Plasma concentrations (upper panels; different ordinates!) and biliary excretion (lower panels) of various amino acids before (open columns) and after nephrectomy (NX; hatched columns).* significant difference between control and NX ($p<0.05$). n = 6 for each amino acid. n.d. not detectable

renal excretion pathway. After bilateral nephrectomy, plasma concentrations of amino acids were changed and two groups of amino acids could be distinguished. In Fig. 2 (upper part) the amino acids are arranged with regard to plasma concentrations of controls. In the group of amino acids with low plasma concentrations (≤ 100 μM) nearly no significant changes occurred after NX (exception: asparagine) whereas, in general, the values of amino acids with higher plasma concentrations (≥ 100 μM) were distinctly diminished after NX. The enhanced plasma concentration of taurine after NX is not surprising because taurine is obligatorily excreted *via* urine (Chesney *et al.* 1989).

The biliary excretion of amino acids was not correlated with their plasma concentrations (Fig. 2, lower part). Tyrosine, glutamic acid, isoleucine, leucine, glycine, alanine, and glutamine were very effectively excreted into bile. On the other hand, the hepatic excretion was very low for β-alanine, carnosine, asparagine, histidine, citrulline, tyrosine, and arginine. The hepatic excretion of most amino acids was not significantly changed after NX. The more effective biliary excretion of taurine correlates with its increased plasma concentration. Surprisingly, the excretion of leucine into bile increased and that of tyrosine decreased.

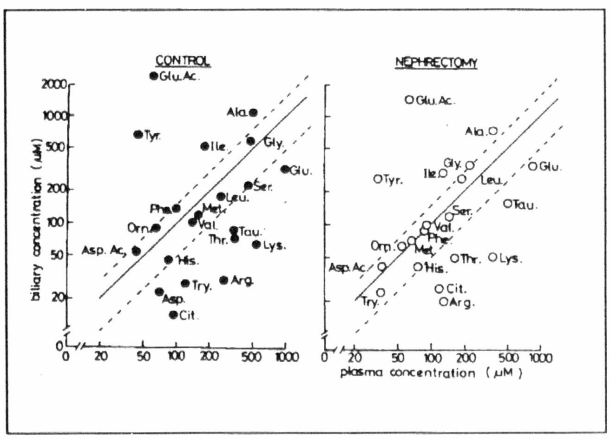

Fig. 3

Relation between plasma concentrations and biliary concentrations of various amino acids in controls and after bilateral nephrectomy. Continuous lines: 100 % correlation. Broken lines: arbitrary variation interval ± 50 %.

According to Brauer (1959), the hepatic excretion of different substances can be differentiated on the basis of their bile to plasma concentration ratios. Substances whose concentrations are significantly higher in the bile than in the plasma are considered to be actively secreted. Furthermore, in the present study the absolute rate of biliary excretion of amino acids (cp. Fig. 2) could be directly calculated and compared in control and NXrats because the concentrations of amino acids were measured in bile samples from controls and from NXrats obtained in comparable collecting periods (546 ± 57 and 655 ± 105 μl/100 g b. wt. per 1 hour, respectively). In Fig. 3, the biliary concentrations of various amino acids are correlated with their plasma concentrations. The amino acids can be divided into three groups:

a) For 10 amino acids the biliary concentrations increase proportionally to their plasma concentrations, if an arbitrary variation interval of ± 50 % is accepted (broken lines).

b) For glutamic acid, tyrosine, isoleucine and alanine, significantly higher concentrations could be measured in the bile than in the plasma and active transport into the bile appears to exist.

c) In 8 cases the biliary concentration of the respective amino acid was distinctly lower than its plasma concentration and active secretion can be excluded.

After NX, this classification remained essentially unchanged. However, as a consequence of diminished plasma concentrations of amino acids (cf. Fig. 2) their biliary concentrations were mostly reduced.

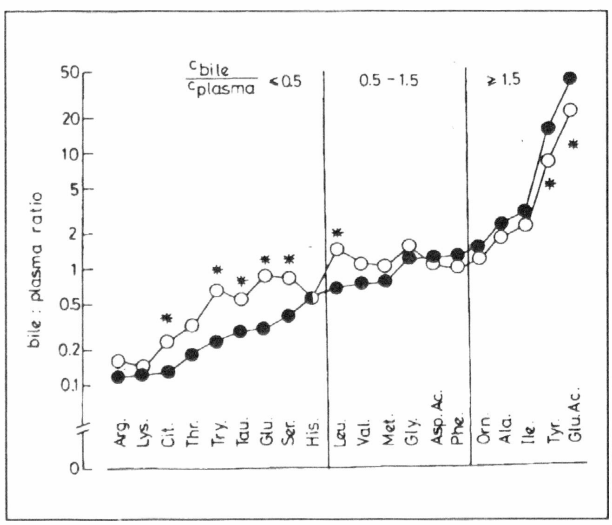

Fig. 4

Influence of bilateral nephrectomy (NX; open circles) on bile to plasma concentration ratios of various amino acids. Black circles: controls. * significant difference between control and NX ($p<0.05$). n = 6 for each amino acid.

To understand the hepatic handling better, the amino acids were arranged in Fig. 4 in the order of increasing bile to plasma concentration ratios in the controls. Low values (≤0.5) indicate active retention mechanisms; high values (≥1.5) indicate active secretion of the respective amino acid into the bile. The three groups mentioned in Fig. 3 can also be distinguished by this mode of presentation. After NX, low ratios increased, high ratios decreased, and intermediate ratios (0.5-1.5) remained unchanged.

Discussion

The liver has long been reported to play an important role in amino acid homoeostasis of the organism (Ballatori *et al.* 1986a,b). Hepatic parenchymal cells contain specific transport mechanisms that coordinate the influx and efflux of the various amino acids (Kilberg 1982, Moseley and Murphy 1989, Berteloot and Maenz 1990). However, attention has been focused nearly exclusively on the metabolism of amino acids in the liver (Jones 1965, Fürst 1989) and their disappearance into bile has not been measured. Both liver and kidney are responsible for maintaining low excretion of amino acids. Thus the reabsorption of amino acids from renal ultrafiltrate amounts to 95-100 % (Silbernagl 1988, Tanaka *et al.* 1989, Zelikòvic and Chesney 1989). Nevertheless, the excretion of amino acids *via* the bile is also very small, although it has not yet been well investigated. Fisher and Kerly (1964) reported amino acid concentrations in rat bile; although amino acids reach the bile primarily as conjugates. In principle, these results are in good agreement with the control values presented in the current study. The differences between the two studies may be caused by different determination methods and by different diets for the animals.

It is known that acute uremia, e.g. after bilateral nephrectomy, influences liver functions and uraemic liver damage develops within 24 hours after NX (Fleck *et al.* in press). Therefore, the hepatic handling of amino acids was investigated in NXrats. In uremia, the biliary loss of amino acids might be expected to increase as a consequence of damaged retention mechanisms. A first result of this series of experiments consisted in the confirmation of other papers reporting reduced plasma concentrations of amino acids in uremia (Salusky *et al.* 1983, Hara *et al.* 1987). This reduction appears to be more marked if the plasma concentration of the respective amino acid is relatively high in the controls. Flügel-Link *et al.* (1983 and 1984) found slightly decreased protein synthesis and increased muscle protein degradation in uraemic rats. Reduction in food intake is not a major factor in the aetiology of the plasma amino acid decrease seen in uremia (Haines *et al.* 1989, Cappelli *et al.* 1990).

Interestingly, Schaefer *et al.* (1989) reported an increased amino acid uptake by the liver after NX. This could explain the higher biliary excretion of amino acids normally prevented from readily entering the bile (bile to plasma concentration ratios ≤ 0.5). Presumably because of the occurrence of uraemic toxins in plasma after NX (Ringoir *et al.* 1988), the barriers normally preventing the passage of highly charged negative ions into the hepatocytes become permeable (Ross *et al.* 1967). This hypothesis is supported by findings of Ballatori *et al.* (1986). They described a canalicular glutamate transport system and suggested that this system may serve to reabsorb this amino acid from bile. After NX this carrier-mediated reabsorption could also be damaged and bile to plasma concentration ratios come near to 1. On the other hand, few amino acids (glutamic acid, tyrosine, isoleucine, alanine) are excreted actively into the bile and their bile to plasma concentration ratios are relatively high (≥ 1.5). For these amino acids it could be shown that their bile to plasma concentration rates are diminished after NX. This could serve as further evidence for uraemic hepatic failure.

Summarizing it can be concluded that:

1. Loss of amino acids *via* bile is very low and retention mechanisms are as effective as those in the kidney.
2. After NX, plasma concentrations of amino acids decrease.
3. In acute uremia the biliary excretion of amino acids is not significantly changed in 19 of 23 cases.
4. Obviously, active hepatic excretion and retention mechanisms appear to be disturbed after NX.

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