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Metabolic alkalosis as driving force for urea synthesis in liver disease: pathogenetic model and therapeutic implications*

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Summary. Despite a marked reduction of the urea cycle capacity, patients with well-compensated chronic liver disease excrete near-normal amounts of urea. Compensation of the urea cycle defect apparently occurs through the activation of liver glutaminase, as suggested by an inverse relationship between the in vitro ureagenic capacity and the flux through glutaminase in liver tissue from patients with a normal, fatty, or cirrhotic liver. In these patients, the flux through glutaminase, as determined in vitro, increases in parallel with the plasma bicarbonate level and plasma pH determined in vivo. In view of this and results from previous studies, the following hypothesis is suggested: The decrease of urea cycle enzyme activities in liver cirrhosis produces metabolic alkalosis due to an impaired bicarbonate elimination. Alkalosis in turn activates and stabilizes hepatic glutaminase and accordingly mitochondrial ammonia provision for carbamoylphosphate synthetase. This results in a compensatory stimulation of the urea cycle flux in the cirrhotic patient to near-normal rates, despite the marked reduction of urea cycle enzyme activity. Accordingly, alkalosis is an important driving force for urea synthesis in the cirrhotic patient. With respect to clinical medicine, attention must be paid to acid-base disturbances in the hyperammonemic patient.

Key words: Urea synthesis – Metabolic alkalosis – Liver cirrhosis – Hepatic encephalopathy – Hyperammonemia

A sophisticated structural and functional organisation of glutamine- and ammonia-metabolizing pathways in the liver acinus has been established in recent years with important consequences for the understanding of ammonium and bicarbonate homeostasis in health and disease (for reviews, see [4, 6, 8, 10, 11, 26]). In liver cirrhosis, even when clinically latent, the capacity to synthesize urea as well as urea cycle enzyme activities are markedly diminished [1, 5, 9, 12–14, 22, 23, 27, 28], and we have ascribed the accompanying mild metabolic alkalosis [3, 9, 18, 19, 24] to a diminished hepatic bicarbonate disposal when the urea synthesis fails [9, 12]. The impairment of the ureagenic capacity by up to 80%, however, contrasts with the clinical condition of the patients with extremely well-compensated liver disease [9]; despite unrestricted protein intake, no hyperammonemia is present in these patients [9]. Obviously, compensatory mechanisms must come into play. One possible site of compensation is the activity of mitochondrial glutaminase in liver. This enzyme plays a crucial role in urea cycle flux control by setting the operational intramitochondrial ammonia concentration (for reviews, see [6, 8]). Here we report on the relationship between alkalosis/hyperbicarbonatemia, glutaminase, and ureagenic capacity. From these and previously published data on humans [9] and studies with rat liver (for review, see [6]), a new hypothesis is presented on the pathobiochemistry of ammonia and acid-base homeostasis in chronic liver disease. New strategies for the treatment of hyperammonemia, as implied by this hypothesis, are discussed.

Methods

The study presented here was performed as part of a previously published study [9]. Criteria on patient selection, study design, liver tissue sampling, and determination of metabolic fluxes in vitro are described there in detail. In brief, liver sam-

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ples obtained during diagnostic laparoscopic biopsy were immediately divided into two parts. One part was used for histological examination, and the other for immediate metabolic examinations. Based on the histology results, three groups were distinguished: normal, fatty and cirrhotic liver. In about 80% of the patients with liver cirrhosis, the disease was due to alcoholism; in the remaining cases it was either due to viral hepatitis or of unknown etiology. Normal and some pathological liver samples were also obtained from patients with malignant disease who underwent diagnostic laparoscopy for staging purposes and whose histological liver abnormality was detected by chance. Only patients without macroscopic or microscopic signs of tumor infiltration and without signs of pulmonary or renal disease were included in the study. All patients with liver disease were in a very well-compensated state and received no diuretic or antacid treatment or dietary restrictions (for detail, see [9]). Informed consent for laparoscopy was obtained from all patients, and the study was approved by the ethics committee of the University of Freiburg. Venous blood samples for the determination of the acid-base status and ammonia were taken without venous stasis after an overnight fast and a 30–60-min bed-rest 1–2 days before or after the laparoscopy, but not on the day of laparoscopy itself in order to avoid any effects of the premedication. Slices of 0.2–0.5 mm thickness were prepared from the liver biopsies and were incubated (10–30 mg tissue/2.5 ml) in bicarbonate-buffered Krebs-Henseleit saline plus L-lactate (4.2 m*M*), pyruvate (0.6 m*M*), and ornithine (2 m*M*). The medium was continuously gassed with a water vapor-saturated O_2/CO_2mixture (95/5, v/v). After equilibration for 10 min, NH_4Cl (10 m*M*) was added, and samples for the determination of urea and glutamine formation were taken at 30-min intervals over a 180-min incubation period. For the determination of glutamine breakdown ("glutaminase flux"), the slices were incubated in the above-mentioned medium plus methionine sulfoximine (0.2 m*M*) and glutamine (0.6 m*M*). The urea formation and glutamine consumption were measured enzymatically as described [2]. The rates of urea synthesis and of glutamine breakdown were constant over the 3-h incubation period and linearly increased with the amount of protein or DNA incubated. The acid-base status was assessed with an automatic blood gas analyzer (ABL 300; Radiometer, Copenhagen).

Data are expressed as means ± SEM. The statistical significance was tested by means of Student's unpaired *t*-test. The correlation coefficients were determined by Pearson's formula.

Results

As reported previously [9], liver specimens from patients with fatty and cirrhotic livers produced urea in vitro at significantly lower rates than those from normal controls. Similar data were obtained in previous in vivo and in vitro studies [1, 3, 5, 9, 12–14, 22, 23, 27, 28]. However, the amount of urea excreted daily into the urine was not significantly affected in patients with a fatty liver (despite a 50% decrease of the in vitro capacity to synthesize urea) and was decreased by only about 30% in cirrhotic patients (despite a 80% decrease of the capacity to synthesize urea in vitro) (Table 1), whereby hyperammonemia was absent. Apparently, compensation of the urea cycle defect occurs in vivo. The possibility that urea cycle enzyme activities are normally overexpressed such that a 50% loss of enzyme activity would be irrelevant is considered unlikely, because the loss of urea cycle capacity in fatty liver is already associated with hyperbicarbonatemia in vivo [9]. However, compensation could occur at the level of the ammonia provision for mitochondrial carbamoylphosphate synthetase, the rate-controlling enzyme of urea

Table 1. Effect of liver disease on urinary urea excretion and the in vitro capacity of liver tissue to synthesize urea from NH_4Cl (10 m*M*)

	Normal liver ($n=11$)	Fatty liver ($n=9$)	Cirrhotic liver ($n=8$)
In vitro capacity for urea synthesis (μmol/mg DNA·h)	14.5 ± 0.8	6.9 ± 0.5	2.3 ± 0.2
Urinary urea excretion in vivo (mmol/24 h)	478 ± 43	435 ± 53	325 ± 31
Plasma NH_4^+ (μ*M*)	22 ± 2	34 ± 3	34 ± 6

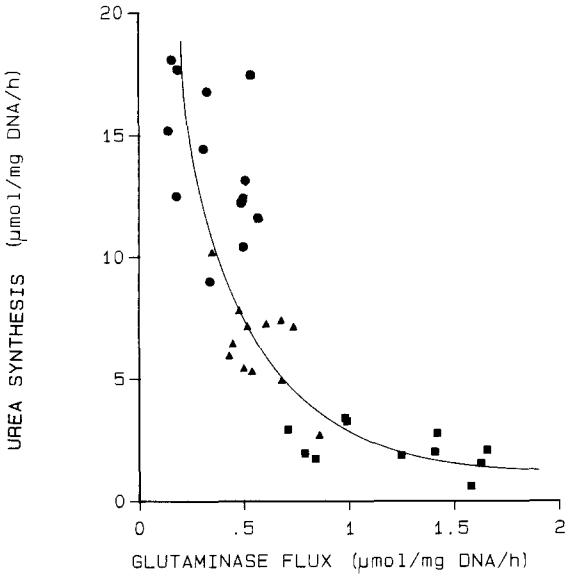

Fig. 1. Relationship between in vitro urea synthesis and glutaminase flux in liver slices from patients with histologically normal (●), fatty (▲), and cirrhotic (■) livers. Data are from 36 patients. For further details see Materials and methods. Pearson's correlation coefficients $r = -0.781$ at a high level of significance ($P < 0.001$)

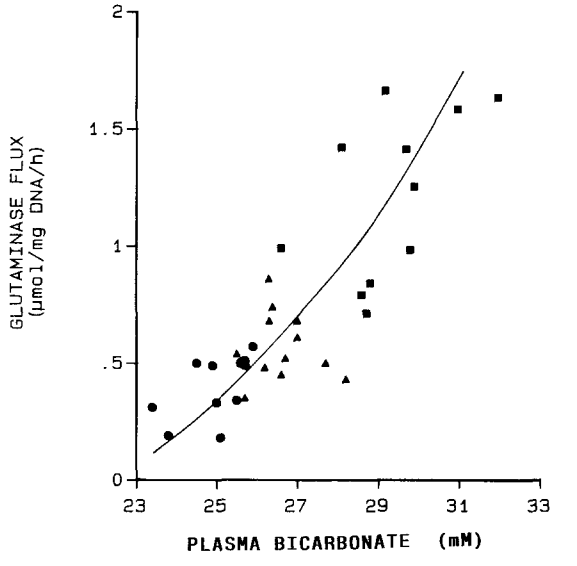

Fig. 2. Relationship between plasma bicarbonate concentration in vivo and the in vitro determined flux through glutaminase in liver slices from patients with histologically proven normal (●), fatty (▲), and cirrhotic (■) livers. Data are from 34 patients. Pearson's correlation coefficient $r = -0.830$ at a high level of significance ($P < 0.001$)

synthesis [16], with its k_m (ammonia) seven–tenfold above the physiological portal ammonia concentration [20, 21]. Liver glutaminase is known to act as a mitochondrial ammonia-amplifying system, and its activity is an important determinant of the urea cycle flux [6, 8]. Thus, stimulation of this ammonia-amplifying system in cirrhosis should elevate the intramitochondrial ammonia level and consequently increase the flux through carbamoylphosphate synthetase. Consequently, a normal in vivo flux through the urea cycle will ensue, although the ureagenic capacity (as assayed under conditions of optimal substrate supply in vitro) is significantly reduced. Indeed, the flux through glutaminase was found to increase with a decreasing capacity for urea synthesis (Fig. 1). The close inverse relationship between the glutaminase flux and the ureagenic capacity was observed regardless of whether reference was made to the amount of DNA (Fig. 1), protein, or liver tissue (not shown) in the incubations. The increase in glutaminase flux in vitro paralleled the in vivo determined plasma bicarbonate level (Fig. 2). Likewise, the in vitro glutaminase flux increased with the in vivo plasma pH [correlation coefficient $r = 0.79$ at a high level of significance ($P < 0.001$)].

Discussion

Hypothesis

Several factors may contribute to the compensatory stimulation of glutaminase when urea synthesis fails. Among them, alkalosis as a short-term activator of both human and rat liver glutaminase [7, 25] requires special attention. Glutamine hydrolysis is extremely sensitive to pH: in perfused rat liver, the flux through glutaminase may increase about three-fold upon raising the extracellular pH by just 0.1 pH unit [7]. Ammonia in the near-physiological concentration range was recently shown to be essential not only for glutaminase induction, but also for protecting the enzyme protein from degradation [14, 15]. Because the short-term activation of glutaminase in alkalosis [7, 25] will keep the mitochondrial ammonia levels high, metabolic alkalosis may also act to stabilize the enzyme by inhibiting its degradation and augmenting its induction.

Taking into account the data presented here and in previous studies [6, 9, 12], the following hypothesis is suggested (Fig. 3). A loss of the urea cycle enzyme activity in liver disease will impair urea synthesis and consequently HCO_3^--disposal from the organism. Accordingly, metabolic alkalosis develops, which activates glutaminase. This results in an increased intramitochondrial ammonia generation and provision for carbamoylphosphate synthetase. Because the flux through carbamoylphosphate synthetase is rate-controlling for urea

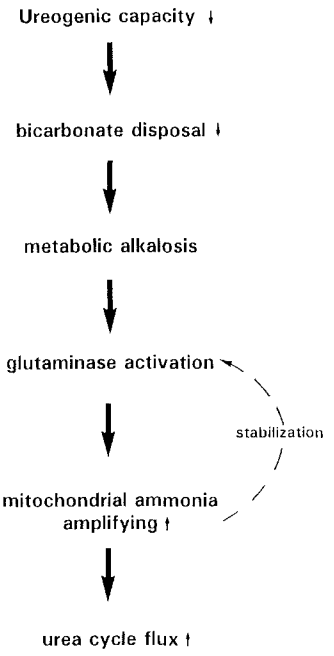

Fig. 3. Hypothesis on the pathobiochemistry of ammonia and bicarbonate homeostasis in liver cirrhosis

synthesis [17] and normally depends on the actual ammonia concentration inside the mitochondria, amplification of the mitochondrial ammonia level by glutaminase is an important determinant of the urea cycle flux [6]. Alkalosis-activation [7, 25] and ammonia-stabilization [15, 16] of glutaminase in chronic liver disease will accordingly enhance the urea cycle flux and counteract its underlying impairment until a near-normal rate of urea synthesis is achieved. The cirrhotic patient approaches a new, albeit more alkalotic steady state, which allows him to dispose of ammonia and bicarbonate via urea synthesis at the same rate as these compounds are generated during protein breakdown. Thus, alkalosis must be viewed as an important driving force for maintaining normal rates of urea synthesis in liver disease by keeping the mitochondrial ammonia amplifier glutaminase active.

Clinical implications

Several implications for the management of hyperammonemic states arise from the pathobiochemical sequence depicted in Fig. 3. First, alkalosis is no longer seen as a precipitating cause of hepatic encephalopathy; its is rather an epiphenomenon of a causally much more relevant disturbance of urea synthesis. Instead, metabolic alkalosis is an important driving force for urea synthesis in the cirrhotic patient, and its correction is expected to worsen the hepatic ammonia detoxication. Second, precipitation of hepatic encephalopathy during sepsis or infection may not only be due to the increased protein catabolism under these conditions, but also to the developing acidosis. Acidosis simply shuts off the ammonia amplifier glutaminase with a consequent inhibition of urea synthesis. Accordingly, the hyperammonemic cirrhotic patient prone to acidosis (due to infection, sepsis, or cardiac insufficiency) is expected to benefit from correction of the acidosis. Here, it should be kept in mind that a pH = 7.4 and a plasma bicarbonate level of 25 m*M* in a normally alkalotic cirrhotic patient may already reflect beginning "acidosis." In clinical routine therapy, metabolic acidosis is usually not corrected unless the base deficit exceeds 10 mval/l. This limit seems too high in the cirrhotic patient. Controlled clinical trials are required to test the efficacy of bicarbonate treatment in the encephalopathic cirrhotic or subpopulations there of.

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Book Reviews

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P. Klose, M. Thelen, R. Erbel: **Bildgebende Verfahren in der Diagnostik von Herzerkrankungen.** Thieme 1991. 244 Pages, 308 Figures, 46 Tables. Hardcover, DM 198,– (ISBN 3-13-747101-X)

This book is a comprehensive review of cardiac imaging written mainly for cardiologists and radiologists. The first of three sections is a concise background chapter on echocardiography, cardiac catheterization, and chest radiography. The second section covers cardiovascular diseases, beginning with acquired valvular diseases and ending with abnormalities of systemic venous connections. Physical findings and hemodynamic changes of each disease are described as well as imaging by conventional techniques: (1) echocardiography (M-mode, B-mode, Doppler, color Doppler and transesophageal recording); (2) chest radiography; (3) cardiac catheterization. Emphasis is put on echocardiography and chest radiography. The third section deals with new methods of cardiac imaging, such as computed tomography (CT), magnetic resonance imaging (MRI) and nuclear imaging. The book is copiously illustrated with echocardiograms, radiographs, CT, and MRI scans. With the exception of some echocardiograms, the images are of excellent quality. The appropriate use of diagrams and tables, its completeness, and the quality of the illustrations are the book's major strengths. This book is an attractive and readable introduction to the diagnosis of cardiovascular diseases. It can be recommended to anyone interested in cardiac imaging.

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