Downregulation in the expression of the serine dehydratase in the rat liver during chronic metabolic acidosis

Inmaculada López-Flores, Juan Peragón, Raquel Valderrama, Francisco J. Esteban, Francisco Luque, M. Ángeles Peinado, Fermín Aranda, José A. Lupiáñez, and Juan B. Barroso. Down-regulation in the expression of the serine dehydratase in the rat liver during chronic metabolic acidosis. Am J Physiol Regul Integr Comp Physiol 291: R1295–R1302, 2006. First published June 22, 2006; doi:10.1152/ajpregu.00095.2006.—Blood pH controls the activity of important regulatory enzymes in the metabolism. Serine dehydratase (SerDH) transforms L-serine into pyruvate and ammonium and is involved in the regulation of gluconeogenesis from serine in the rat liver. In this work, we investigate the effect of chronic metabolic acidosis on the kinetics, specific protein level, tissue location, and mRNA levels of rat liver SerDH. Experimental acidosis was induced in rats by ingestion of 0.28 M ammonium chloride solution for 10 days. Acidosis significantly (P < 0.05) decreased SerDH activity at all substrate concentrations assayed. Moreover, the V_{max} value was 38.50 ± 3.51 mU/mg (n = 7) of mitochondrial protein in the acidic rats and 92.49 ± 6.79 mU/mg (n = 7) in the control rats. Western blot analysis revealed a significant reduction (14%) in the level of SerDH protein content in the rat liver during acidosis. Immunohistochemical analysis showed that SerDH location did not change in response to chronic metabolic acidosis and confirmed previous results on SerDH protein levels. Moreover, the SerDH mRNA level, estimated by RT-PCR, was also significantly 33.8% lower than in control. These results suggest that during experimental acidosis a specific repression of rat-liver SerDH gene transcription could result, lowering the amount and activity of this enzyme. The changes found in SerDH expression are part of an overall metabolic response of liver to maintain acid-base homeostasis during acidosis.

NH₄Cl; reverse transcriptase-polymerase chain reaction; serine catabolism

ACIDOSIS IS A METABOLIC STATE in which, for different causes, blood pH and bicarbonate fall. In these cases, the organism attempts to compensate for these imbalances by increasing the respiratory frequency or adapting its metabolism to facilitate the removal of protons via renal excretion and thus restore the serum-bicarbonate level. For this, major metabolic adaptations occur in liver and kidney. During this situation, liver metabolism shifts to the net release of this amino acid to serum (1, 21, 50). In kidney, the fall in blood pH values intensifies the catabolism of glutamine and gluconeogenesis (26). The increased glutamine catabolism increases the renal excretion of protons in the form of ammonium and gluconeogenesis as one end pathway of the metabolism of the carbon backbone of glutamine. These changes are due to an induction of glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase (PEPCK) during this situation in the kidney (14). Also, coupled with this transformation, serum-bicarbonate levels rise.

Serine dehydratase (SerDH; EC 4.2.1.13) catalyzes the pyridoxal phosphate (PLP)-dependent deamination of serine and threonine to produce pyruvate and α-ketobutyrate, respectively. This is one of the few enzymes that directly releases ammonia from amino acid, and therefore this enzyme could play an important part in the metabolic adaptation of the organism to acidosis. Also, SerDH is involved in the regulation of liver gluconeogenesis from serine in different dietary, hormonal, developmental (46), and pathological (30) states. Its activity tends to be stimulated in gluconeogenic situations (23, 41) and is depressed by high glucose levels in the tissues (24). The changes in SerDH activity under the above-mentioned conditions appears to be triggered by changes in the transcription of the SerDH gene that are mediated by glucagon and cAMP (25), altering SerDH mRNA levels (37). This stimulation is maximal in the presence of glucocorticoids (19). Also, SerDH is considered a marker of liver maturation and is involved in regulating the development of different tissues (48). Previously (30), we have demonstrated that SerDH activity, as well as the protein and mRNA levels, fell in rat liver during chronic liver injury induced by thioacetamide.

Recently, it has been reported that SerDH changes in the rat kidney in response to acidosis (31), and the role of glucocorticoids has been described in stimulating the activity of this enzyme. In this situation, SerDH behaved differently with respect to PEPCK, a key regulatory enzyme of gluconeogenesis.

To elucidate the situation in the rat liver, where SerDH showed the highest activity value and where the greatest amount of amino acid is broken down, we investigated the kinetic behavior, as well as the protein and mRNA level, of SerDH in experimental metabolic acidosis caused by ammonium-chloride intake. We found that in the liver, as opposed to

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the kidney, the level of SerDH activity, protein, and mRNA significantly fell during acidosis, suggesting a regulation of this enzyme at the transcriptional level in this situation. The similarities and differences in the regulation of SerDH and PEPCK described in liver and kidney suggest that its regulation could be coordinated and integrated in this situation by similar stimuli.

MATERIALS AND METHODS

Chemicals. Substrates, coenzymes and other chemical compounds were purchased from Sigma (St. Louis, MO) and Fluka Chemie (Buchs, Switzerland). Immunohistochemistry and electrophoresis reagents were from Bio-Rad Laboratories (Hercules, CA) and Roche Diagnostics (Indianapolis, IN). The β-actin and oligonucleotide primers were synthesized by Promega (Madison, WI) and by Roche Diagnostics, respectively. Other specific reagents and kits were of research grade.

Animals and experimental design. Male Wistar rats were used in all experiments and received humane care in compliance with national and international guidelines (13, 16, 33). The Committee of Bioethics of the University of Jaén reviewed and approved the experiments described in this work. The rats were adapted to laboratory conditions for 2 wk at a constant temperature of 22°C and artificial light from 0800 to 2000. They were given ad libitum access to a standard diet (Panlab, Barcelona, Spain; A04, D.G.P.A. 16867-CAT, 54.5% carbohydrate, 16.2% fish and meat protein, 2.8% fat, 3 kcal/g) and water. They were then separated into two groups of 15 specimens, each group being subdivided into three cages of five rats per cage. One group was given a solution of 0.28 M NH₄Cl (acidosis group) and the other tap water (control group), both administered via water bottles. For 10 days, all of the rats were allowed ad libitum access to the same preexperimental diet and to either water plus NH₄Cl (acidosis group) or water alone (control group), and the consumption was recorded daily (Table 1). Similar periods of time have been used in other works to induce chronic metabolic acidosis (2, 29). Moreover, a third group of five rats starved for 6 days was used as control in the Western blot analyses.

After 10 days of treatment, three rats that had ingested 0.28 M NH₄Cl (one per cage) and three control rats (one per cage) were anesthetized with ether, and the aortic blood-pH value and the serum-bicarbonate concentration were measured to determine the acidosis degree induced by the experimental treatment. SerDH activity was determined using two nonlinear regression analysis programs: Enzfitter (Elsevier Biosoft) and Graphit (Ethosoft software, Microsoft). The protein concentration of the cytosolic extracts was determined using the Bradford method (7).

Western blot analysis. SDS-PAGE and immunoblotting were performed as described by Barroso et al. (3). Samples from high-speed supernatant fractions were heated to 95°C for 3 min in 62 mM Tris·HCl buffer at pH 6.8, containing 2% (wt/vol) SDS, 10% (vol/vol) glycerol, 2.5% 2-mercaptoethanol, 0.045 mM bromphenol blue and 10 mM 1,4-dithiothreitol. Polypeptides were separated by 10% SDS-PAGE using a Bio-Rad Mini-Protean II apparatus and electroblotted onto 0.45-µm polyvinylidene difluoride membrane (Immobilon-P, Millipore, Bedford, MA) using a semidry transfer apparatus with a 1.5 mA/cm² membrane for 150 min in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid and 10% methanol (vol/vol), pH 11.0. The membranes were blocked with 25 mM Tris·HCl, 100 mM NaCl, 2.5 mM KCl buffer (TBS) at pH 7.6, containing 5% nonfat dried milk and 0.05% Tween 20. The blots were then incubated overnight at 4°C with rabbit anti-SerDH antiserum (diluted 1:25,000 in blocking solution). The anti-SerDH antiserum was developed and kindly donated by Dr. H. Ogawa, Department of Biochemistry, Toyama Medical and Pharmaceutical University Faculty of Medicine Toyama. This antiserum has been previously characterized and used in different works (38–40). The blots were washed with TBS buffer containing 0.1% Tween 20. Immunodetection was performed using an enhanced chemiluminescence kit (ECL-Plus, Amersham Pharmacia Biotech, Buckinghamshire, England). The blots were scanned with an AgFA Horizon ultra scanner and photographed and analyzed by videodensitometry using Bio-ID 97 computer software from Bio-Profil.

Immunohistochemistry. The remaining five rats of each treatment were anesthetised by an intraperitoneal injection of equitensin (0.36 mg/kg body wt), and the liver was perfused through the portal vein with 50 ml of 10 mM carboxenated PBS followed by 4% paraformaldehyde in PBS. Fixed livers were removed, cut into 8–10 mm³ cubes and postfixed for 3 h at room temperature with the same fixative. Liver blocks were later immersed in 30% sucrose-0.1 M PB (4°C). Sections of 30 µm were made using a cryostat (2800 Frigocut E, Reicher-Jung, Vienna, Austria). Endogenous peroxidase was inhibited on free-floating sections with 0.03% H₂O₂ in PBS for 30 min, and after several washes, they were incubated overnight at 4°C with rabbit anti-rat SerDH serum diluted 1:5,000 in PBS containing 0.2% Triton X-100. The sections were subsequently washed in PBS and incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA), diluted 1:100 for 1 h, washed, and later incubated with peroxidase-linked avidin-biotin complex at room temperature for 90 min. Peroxidase activity was detected by the nickel-enhanced diaminobenzidine procedure (45), and the sections were then mounted on slides using DPX mountant for histology. Control procedures were

Table 1. Effects of ingestion of 0.28 M NH₄Cl solution on rat body and liver weights, diet and water intake, aortic blood-pH values, and serum-bicarbonate concentration

<table>
<thead>
<tr>
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<th>Control</th>
<th>Acidosis</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>378.32±18.9 (15)</td>
<td>366.03±27.04 (15)</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>15.45±1.07 (15)</td>
<td>13.41±1.35 (15)</td>
</tr>
<tr>
<td>Diet intake, g/day</td>
<td>27.18±0.77 (10)</td>
<td>25.36±0.96 (10)</td>
</tr>
<tr>
<td>Water intake, ml/day</td>
<td>35.03±2.22 (10)</td>
<td>38.48±3.99 (10)</td>
</tr>
<tr>
<td>Aortic blood pH</td>
<td>7.41±0.04 (3)</td>
<td>7.13±0.03 (3)*</td>
</tr>
<tr>
<td>Serum bicarbonate, mM</td>
<td>22.70±1.24 (3)</td>
<td>11.95±0.71 (3)*</td>
</tr>
</tbody>
</table>

Data are means ± SE with the number of values in parentheses. Diet and water intake were expressed as grams of diet or milliliters of water ingested per rat and day during the experimental period. *Significantly different (P < 0.05) when compared to control.
carried out with the primary antibody either being omitted or replaced with an equivalent concentration of preimmune serum.

Determination of SerDH-mRNA levels by RT-PCR. The extraction of RNA and RT-PCR procedure was performed as described by López-Flores et al. (30).

Extraction and isolation of RNA. Total RNA was isolated from rat liver by the modified acid guanidium-phenol-chloroform extraction method (12) using RNAzol B (Cinna Biotex Laboratories, Houston, TX).

Reverse transcription. Samples of 2 μg of total RNA were subject to reverse transcription with oligo(dT) as primer in a medium containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 5 mM MgCl₂, 1 mM of each dNTP, 1.6 μg of oligo(dT)₁₅, 50 units of ribonuclease inhibitor, and 20 units of avian myeloblastosis virus reverse transcriptase (AMV-RT) in a final volume of 20 μl. The reaction mixture was incubated for 1 h at 42°C to synthesize corresponding cDNAs and then heated to 99°C for 5 min.

PCR. The reverse-transcribed mixture was amplified using specific primers for SerDH and β-actin DNA sequences. The oligonucleotide primers were designed from the SDH3 cDNA (36) sequence using the program Oligo 4.1 (National Biosciences, Plymouth, MN). The sense and antisense sequences were: 5'-GCGTAAAATTTGCTGC-3' and 5'-CAGCATCTCTCCACACTT-3'. These sequences anneal at 55.3°C and are the complements of two segments of rat-liver SDH3 probe between the 279–298 and 432–451 positions, respectively. The amplification product was 173 bp in size. Primers for β-actin were used as a positive control for PCR and were designed against a consensus β-actin sequence to amplify a product of 296 bp. The primers were actin sense 5'-TCATGAAATGTGACGTTGCATC-3' and actin antisense 5'-CCTAGAAGCATTTGCGGTGC-3'. These sequences have an annealing temperature of 59.3°C. Sets of primers were chosen with similar annealing temperatures to give clean results on coamplification.

The amplification medium contained 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 2.5 units of Taq polymerase, 1 pmol of each SerDH cDNA primer and 20 μl of RT mixture to a final volume of 100 μl. The reaction mixture was first denatured for 5 min at 95°C and then subject to 30 cycles of amplification in a DNA thermal cycler. Each cycle consisted of a heat-denaturing step at 95°C for 1 min, an annealing step at 60°C for 1 min, and a polymerization step at 72°C for 1.5 min, except for the final polymerization step of the 30th cycle, which was maintained for 5 min. After the first 10 cycles, 1 pmol of β-actin cDNA primer was added to the reaction mixture and 20 additional amplification cycles were completed. To avoid masking the amplification product of the SerDH mRNA sequence when the SerDH and β-actin cDNA primers were added simultaneously, the β-actin primers were added 10 cycles after the SerDH cDNA primers, thus achieving the optimum amplification of both sequences (30).

Two control reactions were set up for each experimental situation: one tube without AMV-RT to check for the presence of any contaminating cDNA template and another without total RNA to detect any contamination in the reaction mixtures. Both controls were consistently negative.

The PCR products were separated by electrophoresis on 4% agarose. DNA was visualized by ethidium bromide using an ultraviolet transilluminator and then photographed. Band intensity was quantified by densitometry assisted by the Bio-1D 97 program.

To demonstrate the identity of RT-PCR products, we made Southern blot analyses using a specific SerDH cDNA probe (SDH3) (36) labeled by random priming with digoxigenin. The DNA fragments from 4% agarose electrophoresis were blotted onto a nylon membrane (Hybond-N⁺, Amersham Pharmacia Biotech, Buckinghamshire, England) by saline capillary transfer and detected using a DIG nucleic-acid detection kit (Roche Diagnostics, Indianapolis, IN).

Statistics. The results are expressed as the means ± SE. Results were tested by one-way ANOVA. The differences between means were analyzed using an unpaired Student’s t-test. Any possibility of cage effect was tested for each parameter, also using the ANOVA analysis. No differences were found between cages of the same experimental group.

RESULTS

The ingestion of 0.28 M of NH₄Cl for 10 days markedly decreased aortic blood-pH values and serum-bicarbonate concentrations (Table 1), indicating that a metabolic acidosis was caused by this treatment, like previous results in other experimental chronic acidosis experiments (2, 15). No significant differences were detected in weight and intake between the two experimental groups.

The kinetic behavior of serDH in the rat liver was examined in cytosolic supernatants by determining its activity at different serine concentrations. A typical hyperbolic curve appeared in both control and acidotic rats (Fig. 1). In both cases, the values of the Hill coefficient showed no evidence of sigmoidicity (Table 2). Vₘₐₓ and specific activity at saturated substrate concentration were about 58% lower in acidotic rats than in control (Table 3, Fig. 1). Similarly, at all substrate concentrations assayed, the specific activity of SerDH in the acidosis group was significantly lower than in control (Fig. 1). The Kₘ values for acidosis was 28.8% higher than in control (Table 2). Catalytic efficiency (Vₘₐₓ/Kₘ) and total activity in the livers of acidotic rats were 67% and 64% lower than in control (Table 2).

Fig. 1. Effect of concentration of l-serine on liver serine dehydratase (SerDH)-specific activity in control (□) and acidotic rats (○). Results are expressed as means ± SE of 7 values. Top: effect of l-serine concentration on SerDH-specific activity are shown. Bottom: double-reciprocal plots are drawn.
The percentage of immunoreactive area per field (mean±SE) found in acidotic rats, although at lower immunoreactivity perivenous areas or in the bile ducts. A similar distribution was seen in the acinus (Fig. 3). Immunoreactivity was not detected in the hepatocytes located at the periportal level of the hepatic lobule of the control rat.

The specific SerDH immunoreaction product was detected only in the cytosolic supernatants of a 6-day starved rat (lane 1), acidotic rat (lane 2), and control rat (lane 3) were electrophoresed (30 μg per lane) and then blotted to polyvinylidene difluoride. The 34.1-kDa polypeptide, corresponding to a monomeric form of the enzyme, was detected with specific polyclonal anti-SerDH rabbit serum. The immunoblot is representative of five determinations.

Results are expressed as means ± SE of 7 values. Specific activity was determined at 0.5 M of L-serine. \(K_m\), Michaelis constant for L-serine; \(V_{max}\), maximum velocity. The activity ratio \(V_{0.1M}/V_{max}\) was determined as the ratio of the specific activity at 0.1 M of L-serine and \(V_{max}\). Catalytic efficiency \(V_{max}/K_m\) is expressed as milliunits (mU) of enzyme per milligram of protein per millimoles. Total activity is expressed as enzyme units in the whole organ. SerDH, serine dehydratase. *Significantly different \((P < 0.05)\) when compared to control.

**Table 2. Effect of chronic acidosis on the kinetic behavior of serDH in the rat liver**

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<tr>
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<th>Control</th>
<th>Acidosis</th>
</tr>
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<tbody>
<tr>
<td>Specific activity, mU/mg protein</td>
<td>42.64±3.13</td>
<td>17.75±1.62*</td>
</tr>
<tr>
<td>(K_m), mM</td>
<td>123.3±2.6</td>
<td>158.8±4.9*</td>
</tr>
<tr>
<td>(V_{max}), nmol/min</td>
<td>92.49±6.79</td>
<td>38.50±3.51*</td>
</tr>
<tr>
<td>Hill coefficient, h</td>
<td>1.00±0.07</td>
<td>0.98±0.00</td>
</tr>
<tr>
<td>Activity ratio, (V_{0.1M}/V_{max})</td>
<td>0.40±0.03</td>
<td>0.35±0.03</td>
</tr>
<tr>
<td>Catalytic efficiency, (V_{max}/K_m)</td>
<td>0.76±0.08</td>
<td>0.25±0.02*</td>
</tr>
<tr>
<td>Total activity, Units</td>
<td>43.68±3.21</td>
<td>15.80±1.44*</td>
</tr>
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</table>

**DISCUSSION**

In humans, important chronic pathologies such as chronic renal failure, sepsis, severe trauma, or diabetes mellitus are characterized by chronic metabolic acidosis, which is involved in the lethal effects of these pathologies, as well as in increased protein-degradation rates, growth delay, body-weight loss, and alterations in mineral metabolism (2, 8). The oral administration of 0.28 M ammonium chloride for 5–10 days is an experimental model that induces chronic acidosis in rats and other rodents (2, 8). The blood pH measured in this experimental model is similar to those found in the above pathological situations. In other studies, acute treatments of high concentrations of acids such as HCl have been applied via intragastric catheter or intravenous injection (43).

In our work, biochemical and molecular analyses revealed a significant fall in the serine-dehydratase expression in rat liver during experimental chronic acidosis. This metabolic adaptation is part of an overall metabolic response of liver to maintain the acid-base homeostasis during acidosis. In this situation, a high plasma level of ammonium (one of the products of the reaction catalyzed by SerDH) results, as does an inhibition of

**Table 3. Relative quantification of hepatic SerDH protein and mRNA levels in control and acidotic rats by densitometry of Western blot analysis and RT-PCR products**

<table>
<thead>
<tr>
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<th>SerDH protein</th>
<th>SerDH mRNA</th>
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<tr>
<td></td>
<td>Control</td>
<td>Acidosis</td>
</tr>
<tr>
<td>Arbitrary Units</td>
<td>955.0±37.7</td>
<td>821.3±38.3*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE of 5 values. *Significantly different \((P < 0.05)\) when compared to control. In the quantification of RT-PCR products, the results are expressed as arbitrary units of integrated optical density, the value of SerDH RT-PCR product was normalized with the value of \(\beta\)-actin.

Fig. 2. Immunoblot of SerDH of liver in acidotic rats. Protein from liver cytosolic supernatants of a 6-day starved rat (lane 1), acidotic rat (lane 2), and control rat (lane 3) were electrophoresed (30 μg per lane) and then blotted to polyvinylidene difluoride. The 34.1-kDa polypeptide, corresponding to a monomeric form of the enzyme, was detected with specific polyclonal anti-SerDH rabbit serum. The immunoblot is representative of five determinations.

34.1 kDa
the transport into the hepatocyte of some amino acids (4). Both facts could explain the lower expression levels of SerDH during acidosis, which could lead to an inhibition of gluconeogenesis from the serine produced in this situation.

Our results showed that in acidic rats the hepatic SerDH activity at all substrate concentrations decreased 58% with respect to control. The results of the Western blot analysis and immunohistochemistry, using specific antisera anti-rat-SerDH showed a lower amount of immunoreactive protein in liver of acidic rats. Moreover, the RT-PCR results showed a fall in the SerDH mRNA level with respect to control. These results suggest that SerDH expression in the liver is downregulated at the transcriptional level under chronic metabolic acidosis in rats.

The reduction detected in activity, protein, and mRNA level of SerDH in rat liver during chronic metabolic acidosis cannot be due to differences in the supply of nutrients or metabolic fuels. SerDH activity and protein reportedly increase significantly under gluconeogenic conditions, such as starvation (25), a free-protein diet, or physiological conditions of high concentrations of exogenous protein (35). SerDH is depressed under high levels of glucose for the tissues (24). This stimulation is mediated by glucagon via cAMP (25) and glucocorticoids (37) that have a permissive effect on it (35). Fasting and experimental diabetes are two situations that alter the level of available cellular fuels and cause metabolic acidosis. Serum pH values in both situations could be lower than 7.2 under severe conditions (28). In contrast to our results, SerDH gene expression in other studies was reportedly boosted in both of these situations (41, 46). The likely reason for this different behavior is that chronic metabolic acidosis induced by ammonium chloride does not change nutrient availability; that is, the ingested amount of carbohydrate, lipids, and proteins remain the same, and therefore, no differences occur in the glucose or cAMP level that could induce this gene in the case of fasting and diabetes. In this sense, it is noteworthy that the release of glucocorticoids during metabolic acidosis has been well established (5, 46), although in the liver, glucocorticoid itself does not induce SerDH in vivo but rather has a permissive effect on transcription by stimulating the action of glucagon via cAMP (47).

The above results indicate that glucose or gluconeogenic substrate availability can be an essential stimulus for the SerDH. With these antecedents, and taking into account that our experimental conditions did not involve a differential supply of nutrient between treatments, we conclude that there must be other stimuli responsible for the regulation found in our experiment. Therefore, we suggest that the changes associated with the ammonium-detoxification processes are responsible for the fall in activity, protein levels, and SerDH mRNA. In this respect, it has also been demonstrated that blood pH regulates urea synthesis and gluconeogenesis (26). At acidic pH values, urea synthesis, and gluconeogenesis are strongly depressed and not effectively stimulated by the prime regulatory hormones of these pathways (26). Investigating this decrease, Boon and Meijer (4) demonstrated an inhibition of transport of some amino acids to the interior of the hepatocyte, decreasing the intracellular concentration of amino acids.

Metabolic acidosis stimulates the degradation of muscle proteins and amino acids (34). In this situation, the kidney removes the excess of acids increasing ammonium excretion. The liver also has an important function in regulating the acid-base balance in this situation. In this tissue, the net nitrogen balance is due to two simultaneous processes: urea synthesis and glutamine production. In a normal situation, the prime pathway to nitrogen excretion is urea synthesis, but during acidosis, an important metabolic change occurs, in which the urea synthesis is inhibited and the glutamine metabolism changes, leading to the net release of this amino acid into blood (1, 21, 50). In rat liver, urea synthesis and glutaminase activity is located primarily in periportal hepatocytes, whereas glutamine synthetase and the glutamate-transport system are...
Gene expression. It is demonstrated that the activity and expression of SerDH is depressed so as not to contribute to a higher production of this toxic compound. Therefore, in one situation in which excess serum ammonium and that SerDH expression was presume that the synthesis of glutamine was supplied with a high ammonium concentration exists, the activity and enzymatic properties, one of which, urea synthesis, is a system with a low affinity and a high capacity, whereas the other, glutamine biosynthesis, is a high-affinity system. Urea synthesis, in contrast to glutamine synthesis, is a prime pathway for the removal of metabolic bicarbonate (22). During acidosis, a defect in serum bicarbonates exists, so that the change in the ammonium detoxification from urea synthesis to glutamine synthesis can have an important function in the role of liver as the organ responsible for maintaining blood-pH homeostasis.

Ureagenesis and gluconeogenesis from serine showed a tissue location and analogous behavior (9, 22, 38). The key regulatory enzymes of ureagenesis and gluconeogenesis are located in the same zone of hepatic acinus, showing a parallel behavior of these pathways. Therefore, in the situations of high blood-urea concentrations, gluconeogenesis, in general (18), and from serine, in particular (27), are stimulated; meanwhile, when urea production is inhibited, gluconeogenesis (9) and even serine dehydratase also decrease. This relationship leads us to assume some degree of channeling between substrates and initial products of both pathways; for example, the carbon backbone of amino acids that give up their NH₃ group for urea synthesis is channeled to glucose production. It appears that gluconeogenesis regulation is maintained by the regulation of urea synthesis. In this sense, it should be considered that one of the end products of SerDH activity is ammonium. SerDH is one of the few enzymes that produce ammonium as a consequence of its activity. Snell (46) proposed that SerDH is a terminal-deamination system for a given number of amino acids, although the relatively low enzyme affinity could be a problem for this function. Therefore, in one situation in which a high ammonium concentration exists, the activity and enzyme amount produced was lower than in control rats. We presume that the synthesis of glutamine was supplied with excess serum ammonium and that SerDH expression was depressed so as not to contribute to a higher production of this toxic compound.

The results in the present work and a previous one (31) demonstrate that the activity and expression of SerDH is differentially regulated in the rat liver and kidney in response to metabolic acidosis induced by ammonium chloride. Here, we demonstrate that, in the liver, the protein and mRNA levels significantly fall, while Masuda et al. (31) showed that a significant glucocorticoid-dependent induction of the enzyme occurs in kidney. This differential regulation enables the integration of SerDH regulation with the overall metabolic changes triggered in each tissue in these situations. In the liver an inhibition of gluconeogenesis and ureagenesis results (9), coinciding with a fall in the SerDH level, while kidney undergoes renal gluconeogenesis, which is maintained partly by a rise in SerDH levels. This differential response demonstrates the existence of a selective expression of SerDH gene in both tissues, where there must be different elements or factors that regulate the transcription of the gene. In this sense, the expression of PEPCK, a regulatory enzyme of gluconeogenesis and glycero-gensis in different rat tissues (6, 20) showed a behavior similar to that described for SerDH. Acidosis specifically stimulated renal but not hepatic transcription of the gene (32), whereas glucocorticoids controlled both hepatic and renal PEPCK-C gene transcription (20). The regulatory elements involved in this selective mechanism of activation of PEPCK transcription in this situation in both tissues are not completely clear. In experiments made in PK1-derived cells, it has been demonstrated that this response involves promoter regulatory elements related to cAMP (CRE), hepatic nuclear factor (HNF)-1, and P3 (II) elements (10). Nevertheless, in the rat kidney, the regulation of gene-promoter activity in response to acidic pH remains unclear. It appears that nuclear receptors modulate hepatic and adipocyte PEPCK-C gene expression, although it also seems that acidosis and nuclear receptors stimulate PEPCK gene transcription independently, despite that both stimuli require intact HNF-1 binding sites (11). Elements similar to that described for PEPCK could be responsible for the regulation of SerDH gene expression in rat liver and kidney during chronic metabolic acidosis. This acts in coordination with other signals produced by other nutritional and hormonal stimuli generated in different acidosis situations—in specific, hormonal changes associated with fuels at the cellular level existing in this situation. This mechanism will be the aim of future investigations.
REFERENCES

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