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Exposure of Perfused Liver to Hypotonic Conditions Modifies Cellular Nitrogen Metabolism

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Isolated livers were exposed to hypotonic perfusates. As shown previously, this hypotonic challenge leads to initial cell swelling, followed by volume regulatory ion fluxes, largely restoring cell volume within approximately 6 min. However, the hepatocyte is left in an altered metabolic state, which is characterized by marked stimulation of hepatic glutamine uptake and degradation and transient release of glutamate from the liver. Urea formation from glutamine and alanine is stimulated, whereas hepatic ammonia uptake and utilization for urea and glutamine synthesis decreases. These observations reveal a hitherto unrecognized factor modulating hepatic function during intestinal absorption.

Key words: cell volume, glutamine, ammonia, urea synthesis, metabolic regulation

During the course of intestinal absorption, liver cells are exposed to hypotonic extracellular fluid. Furthermore, cellular concentrative uptake of organic substrates such as amino acids leads to an osmotic water shift across the cell membrane, requiring mechanisms of regulatory cell volume decrease. As cells from a variety of tissues, liver cells are indeed capable to regulate their volume in hypotonic media [1–5]. If the cells are exposed to hypotonic extracellular fluid, the cells initially swell by uptake of water, but within minutes display a regulatory volume decrease to close to their original volume [2,5]. As in other tissues [6–18], this regulatory volume decrease is accomplished in large part by release of intracellular potassium [1–5].

In euryhaline invertebrate cells, intracellular amino acids are utilized to compensate partially for alterations of extracellular osmolarity [for review, see 19]. Furthermore, the release and/or degradation of intracellular amino acids participates in regulatory volume decrease of Ehrlich ascites tumor cells [20–22] and cells of kidney medulla [23].

The present paper was designed to test whether or not the volume regulatory response involves alterations of nitrogen metabolism in liver. To this end, the effect of hypotonic extracellular fluid on metabolism of glutamine, glutamate, alanine, ammonia, and urea was tested in livers perfused in a nonrecirculating system [24,25].

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MATERIALS AND METHODS

Livers of male Wistar rats (140–250 g body weight), fed ad libitum on stock diet (Altromin), were perfused as described previously [24,25] in an open (nonrecirculating) system at 37°C.

The ion composition of the isotonic perfusate was (in mmol/L) 116 NaCl, 5.9 KCl, 25 $NaHCO_3$, 1.25 $CaCl_2$, 1.18 $MgCl_2$, and 1.23 Na_2HPO_4. Solutions were equilibrated with 96% O_2 and 4% CO_2 (pH 7.55). The osmolarity of the solutions has been checked by freezing-point depression. Osmolarity was reduced (hypotonic) by omission of 40 mmol/L NaCl from the perfusate. The other composition of the portal venous perfusate and the perfusion flow rate (approximately 4 ml g^{-1} min^{-1}) were kept strictly constant throughout the individual perfusion experiments. If applicable, arithmetic means are given ± SEM and paired or unpaired *t*-test performed for statistical analysis; $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In the absence of net water movements, any uptake and release of substances by the perfused liver is reflected by the respective differences of concentrations between influent portal venous perfusate and effluent hepatic venous fluid. In steady state, the potassium activity is identical between influent perfusate and effluent fluid (Figs. 1–3). Sudden exposure to hypotonic perfusate by reduction of perfusate NaCl concentration leads to a biphasic increase of effluent potassium activity (Figs. 1–3). The first peak is created by the uptake of solute-free water by the liver cells, the second peak by volume regulatory release of cellular potassium [5]. Exposure to hypotonic perfusates is paralleled by an alkalinization of the venous effluent, which is sustained in livers from fed animals (Figs. 1, 3) and only transient in livers from fasted animals (Fig. 2). The increased effluent pH reflects reduced acid release by the liver. In a previous study, the sustained alkalinization has been shown to be paralleled by reduced lactate and pyruvate release, due to reduced cellular glycogen degradation [5]. In livers of fasted animals, the cells are probably glycogen depleted. Accordingly, the formation of lactate and pyruvate is minimal during isotonic conditions and cannot be reduced further during exposure to hypotonic conditions.

In the presence of exogenous ammonia and glutamine, both are taken up (1,471 ± 56 nmol/min g liver and 139 ± 11 nmol/min g liver, respectively), and the livers release both glutamate (57 ± 4 nmol/min g liver) and urea (1127 ± 35 nmol/min g liver). Thus, urea is formed mainly from ammonia. Sudden exposure to hypotonic perfusates leads to a biphasic decrease of glutamine concentration, a transient increase of glutamate concentration, a sustained increase of ammonia concentration, and a biphasic decrease of urea concentration in the venous effluent (Fig. 1). These concentration changes cannot be explained by the water movements but reflect alterations of cellular uptake or release, respectively. Accordingly, exposure to hypotonic perfusates leads to a biphasic stimulation of glutamine uptake, a transient stimulation of glutamate release, a decrease of ammonia uptake, and a biphasic decrease of urea formation.

In the absence of exogenous ammonia, the formation of urea is clearly reduced (686 ± 43 nmol/min g liver) even in the presence of alanine and increased glutamine concentration and even if the uptake of amino acids into the liver has been stimulated by prior 24 h starving of the animal. The uptake of alanine approached 884 ± 70 nmol/min g liver and of glutamine 134 ± 20 nmol/min g liver (Fig. 2). Ammonia (444 ± 44

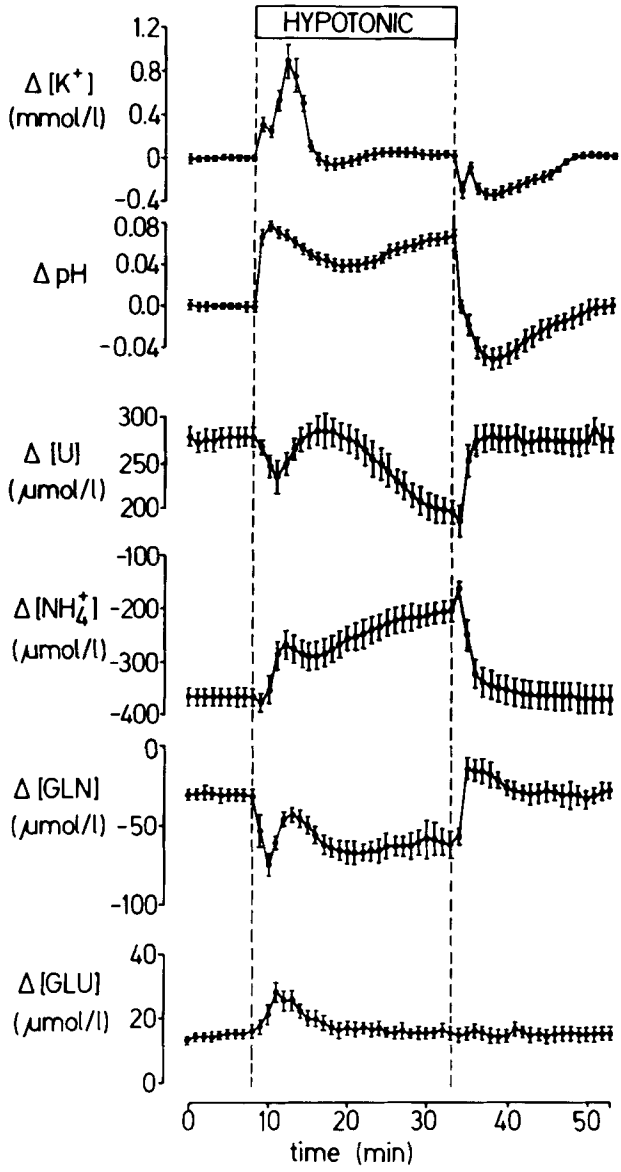

Fig. 1. Effect of hypotonic challenge in the presence of exogenous glutamine and ammonia. Differences between portal venous perfusate and venous effluent of potassium [K^+], urea [U], total ammonia (ammonium ions + ammonia) [NH_4^+], glutamine [GLN], and glutamate [GLU] concentration as well as change of pH in venous effluent (arithmetic means ± SEM, n = 5). The perfusate contained 0.6 mmol/L glutamine and 0.5 mmol/L ammonia, but no urea and no glutamate. Glutamine synthetase was inhibited by 0.2 mmol/L methioninsulfoximine [25]. The livers were from fed animals.

nmol/min g liver) and glutamate (18 ± 11 nmol/min g liver) are formed and released by the liver. Sudden exposure to hypotonic perfusates leads to an increase of effluent urea and ammonia concentration, a biphasic decrease of glutamine concentration, a transient decrease of alanine concentration, and a transient increase of glutamate concentration in the effluent, reflecting increased formation of both urea and ammonia, a biphasic

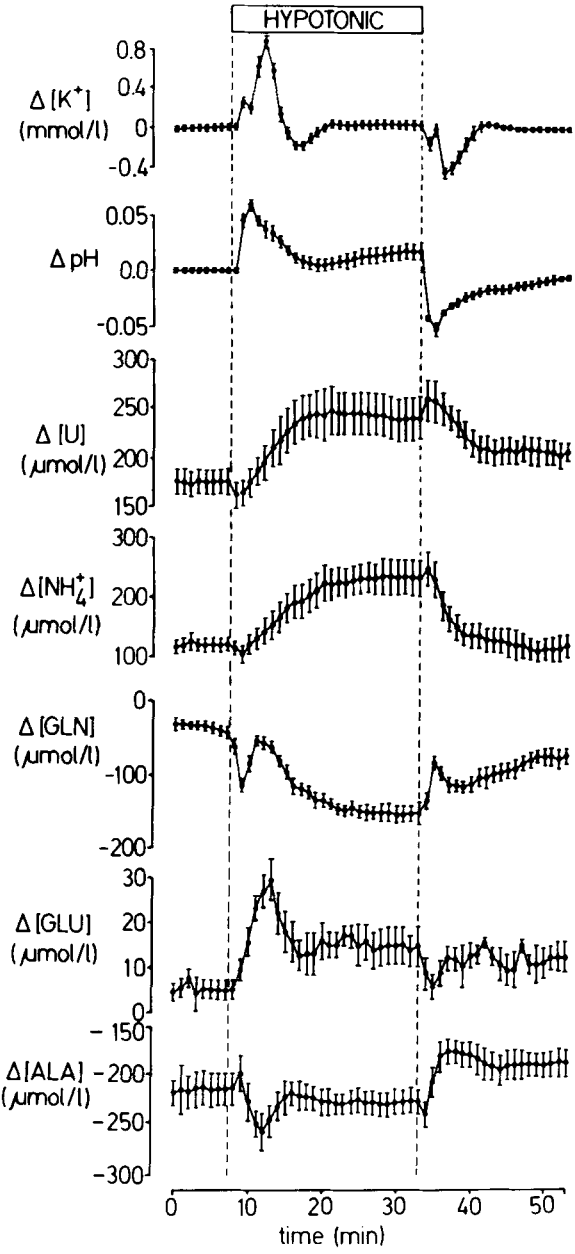

Fig. 2. Effect of hypotonic challenge in the presence of exogenous alanine, glutamine, and glutamate, but not of exogenous ammonia. Differences between portal venous perfusate and venous effluent of potassium [K^+], urea [U], total ammonia [NH_4^+], glutamine [GLN], glutamate [GLU], and alanine [ALA] concentration as well as change of pH in venous effluent (arithmetic means ± SEM, n = 5). Alanine (1 mmol/L), glutamine (1 mmol/L), glutamate (0.1 mmol/L), and methioninsulfoximine (0.2 mmol/L), but not urea or ammonia were added to the portal venous perfusate. The livers were from 24 h starved animals.

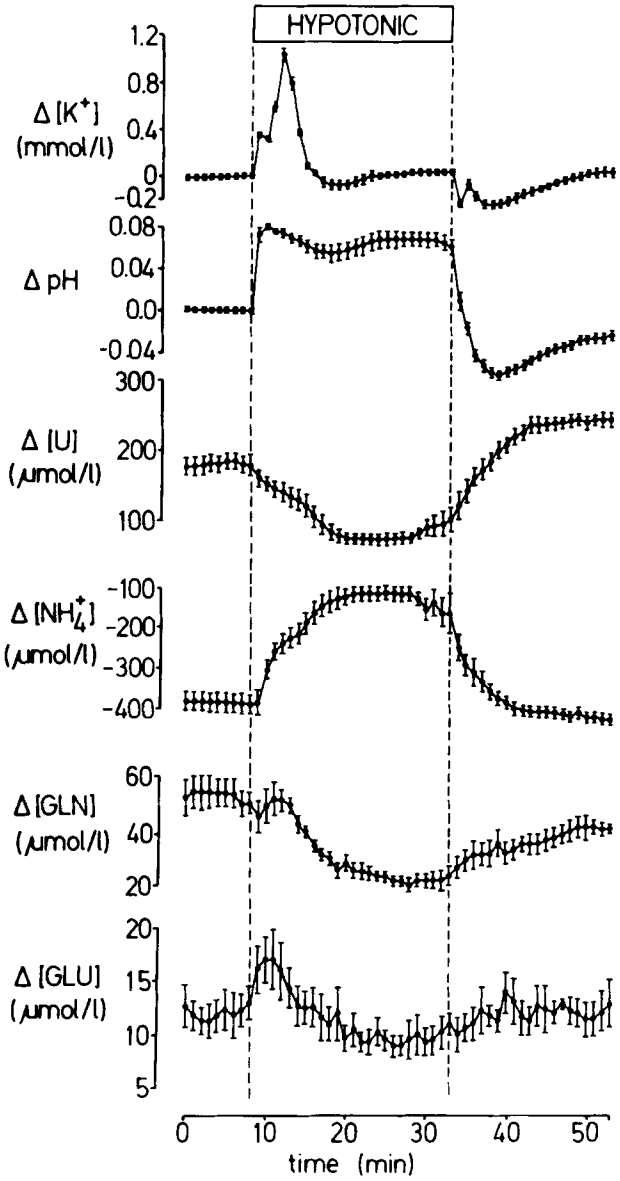

Fig. 3. Effect of hypotonic challenge in the presence of exogenous ammonia, but not of exogenous amino acids. Differences between portal venous perfusate and venous effluent of potassium [K^+], urea [U], total ammonia [NH_4^+], glutamine [GLN], and glutamate [GLU] concentration as well as change of pH in venous effluent (arithmetic means ± SEM, n = 5). Ammonia (0.5 mmol/L) but not urea, amino acids, or methionine-insulfoximine was added to the portal venous perfusate. The livers were from fed animals.

stimulation of glutamine uptake, as well as a transient stimulation of glutamate release and alanine uptake.

In the absence of exogenous amino acids and the presence of ammonia, the livers avidly take up ammonia (1,526 ± 86 nmol/min g liver), and urea formation by the liver is significantly less (764 ± 51 nmol/min g liver) than in the presence of exogenous

ammonia and glutamine. The cells release small amounts of both glutamine (215 ± 28 nmol/min g liver) and glutamate (50 ± 9 nmol/min g liver). Sudden exposure to hypotonic perfusates leads to a marked decrease of urea concentration and a marked increase of ammonia concentration in the venous effluent (Fig. 3), reflecting a decrease of ammonia uptake and urea formation by the liver. Glutamine release is decreased and glutamate release transiently enhanced (Fig. 3).

The present observations show, for the first time, that hypotonic challenge may stimulate cellular uptake of amino acids and modify ammonia transport and metabolism. The stimulation of glutamine uptake and utilization may in part be the result of mitochondrial swelling [26,27]. In contrast to urea formation from glutamine, urea formation from exogenous ammonia is inhibited. In the presence of exogenous ammonia, the influence of ammonia prevails, and hypotonic challenge decreases urea formation. In the absence of ammonia, the increased glutamine uptake not only accounts for the increased urea formation, but also for the increased formation of ammonia. It is noteworthy that the alterations in glutamine and ammonia uptake persist throughout exposure of the liver to hypotonic perfusates (i.e., in these experiments, 24 min), even though volume regulatory potassium release is virtually complete within 6 min. In livers from fed animals, the alkalinization of the venous effluent is similarly sustained, reflecting persisting inhibition of acid production by the liver. Thus, liver cell metabolism does not return to its original state, even after seeming completion of cell volume regulation. The original state, however, is eventually regained after reexposure to normotonic extracellular fluid. Thus, the effects of transient exposure to hypotonic extracellular fluid are fully reversible (Figs. 1–3).

In conclusion, exposure of isolated perfused liver to hypotonic perfusates leads not only to volume regulatory ion fluxes but, in addition, to profound alterations of liver cell metabolism, which may be an integral part of cellular volume control.

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