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Ketone bodies promote a rapid rise in glutamate efflux from the isolated perfused rat liver without altering the rate of glutamine production

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Summary. Livers of starved (48 hr) male Wistar rats were perfused in a non recirculating manner with a near physiological mix of ammonium, lactate, ornithine and pyruvate in Krebs buffer. The addition of ketone bodies (3-DL-hydroxybutyrate [B OHB] 2–30 mM or lithium-acetoacetate (15 mM) to the perfusate resulted in a rapid rise in the efflux of glutamate from the liver (five times above basal). This was not seen with control solutions (sodium chloride or lithium chloride). The increased efflux was sustained for the duration of the addition of the ketone bodies (7 min), was rapidly reversible and dose dependant. Glutamine export rates were not altered, suggesting that either the glutamate originated from cells not responsible for glutamine synthesis or that this glutamate was superfulous to the requirement of glutamine synthesis. There was no evidence that the lactate transporter was involved in the entry of lactate into perivenous hepatocytes for glutamine synthesis; lactate presumably entering the hepatocyte by an alternative pathway, probably non-ionic diffusion.

Keywords: Amino acids – Hydroxybutyrate – Acetoacetate – Glutamine – Glutamate – Liver

Introduction

The perfusion of isolated rat livers results in a rapid efflux of glutamate and glutamine over the first few minutes until a base line rate of production and release is established. The quantity of glutamine or glutamate released depends upon the nutritional state of the animal and the substrates included in the perfusing media (Lund, 1971). It was recently reported (Haussinger et al., 1989) that the addition of certain anions to the perfusate resulted in the stimulation of glutamate release, perhaps mediated by an anion counter transporter (Haussinger et al., 1989).

Under similar experimental conditions ketone bodies are known to inhibit the hepatocyte plasma membrane lactate transporter (Metcalf et al., 1986). The distribution of the hepatic lactate transporter along the hepatic acinus is not known. In an attempt to alter the rate of glutamine or glutamate metabolism by interfering with the lactate transporter or by the proposed anion-counter transporter mechanism ketone bodies were added to the perfusion of isolated rat livers.

Drawing upon knowledge of the functional heterogeneity of the hepatic acinus it is known that urea synthesis and glutaminase are present in the periportal hepatocytes whereas glutamine synthetase is found only in perivenous hepatocytes (Haussinger et al., 1984). Any changes demonstrated in glutamate or glutamine metabolism occuring within the liver need to be ascribed to the correct compartment.

Materials and methods

Animals

Male 48 hr starved Wistar rats approx 175 g were used.

Perfusion technique

Isolated liver perfusions were established as previously described (Cohen et al., 1973), except that a non recirculating, erythrocyte and albumen free perfusion media was used and flow rates correspondingly increased (1 ml/10 g rat weight/min). The basic perfusion media was Krebs bicarbonate buffer, equilibrated with 95%O_2:5%CO_2, pH 7.4 (Haussinger, 1987). Hepatic venous effluent PO_2 was always >10 kPa. To the perfusate was added NH_4Cl 0.6 mM, lactate 2 mM, pyruvate 0.3 mM and ornithine 0.1 mM. Perfusions were equilibrated for 30 mins before timed, paired samples (1 min) of perfusate and hepatic venous effluent were obtained for estimation of pH, PCO_2, PO_2, glutamate and glutamine concentrations.

When sodium 3-DL-hydroxybutyrate or lithium acetoacetate were infused, this was done by infusion pump into the portal venous line at a rate calculated to produce initial concentrations of 30 mM (15 mM D-B OHB) and 15 mM respectively. Control infusions were performed using similar concentrations of NaCl or LiCl. Subsequent infusions were performed on different livers at progressively lower concentrations of B OHB or NaCl to construct a dose dependant curve.

Assays

The perfusate was collected into ice-cold perchloricacid 3 M (10 ml:0.6 ml), and kept frozen. Concentrations of glutamine and glutamate were measured by standard enzymatic techniques based on the procedures described in (Bergmeyer, 1974). pH and PCO_2 were measured on samples of perfusate using a IL 1304 pH/blood gas analyser.

Chemicals

All reagents were of analytical grade and obtained from Sigma, Enzymes were obtained from BCL, Boehringer Mannheim.

Results

Having established a basal rate of glutamine and glutamate production during perfusion for 30 min the addition of, 3-DL-hydroxybutyrate (30 mM B OHB)

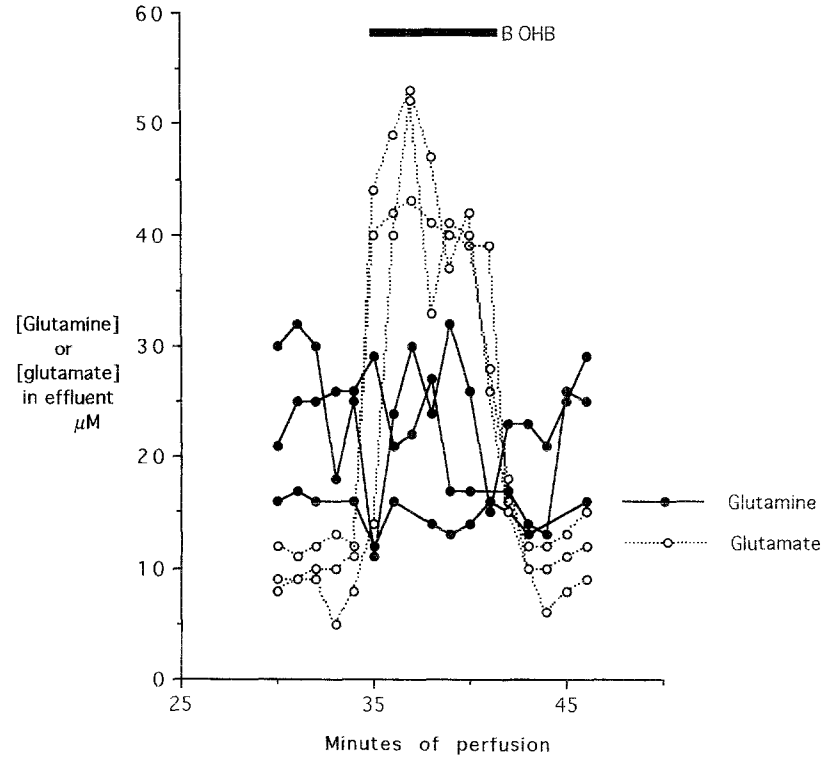

Fig. 1. Representative figure of three isolated perfusions carried out in the presence of lactate 2 mM, ammonium 0.6 mM, ornithine 0.1 mM and pyruvate 0.3 mM. Following an equilibration period of 30 mins 3-DL-hydroxybutyrate 30 mM, (B OHB), was added via an infusion pump to the perfusate for 7 mins

or acetoacetate (15 mM), to the perfusate over a fixed period (7 min) failed to change the rate of glutamine synthesis but resulted in a rapid (<1 min) and reversible efflux of glutamate from the liver. This depletion of hepatic glutamate did not produce a fall in the rate of glutamine production (Figs. 1 and 2), over the time course observed. The glutamate efflux terminated abruptly at the end of the addition of the ketone bodies, (<1 min). No change in the rates of efflux were observed from livers perfused with the addition of control substances (NaCl or LiCl) Figs. 3 and 4.

Figure 5 demonstrates the maximum rise in glutamate efflux observed during the addition of B OHB above the mean rate observed during the preceding, basal, five minutes. Each point represents the perfusion of a single liver with the concentration of added B OHB during that perfusion shown on the horizontal axis. Insufficient points prevent the accurate calculation of the $Km_{0.5}$ and Vmax for B OHB as has been done for benzoate (Haussinger et al., 1989).

Discussion

During experiments infusing hydroxybutyrate or acetoacetate we noted a marked efflux of glutamate, five times greater than basal when [D-beta-

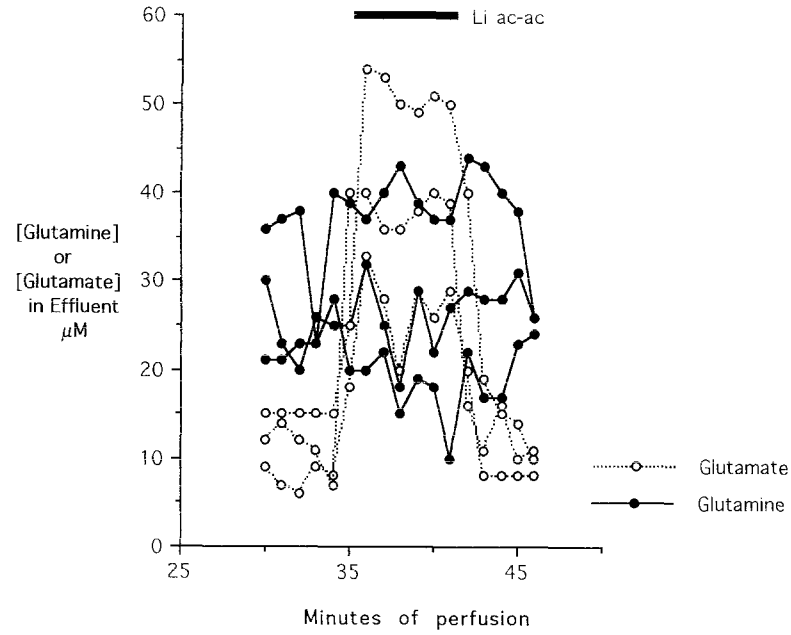

Fig. 2. Three isolated liver perfusions carried out under conditions as described in Fig. 1. With the addition of lithium acetoacetate (15 mM, Li ac-ac)

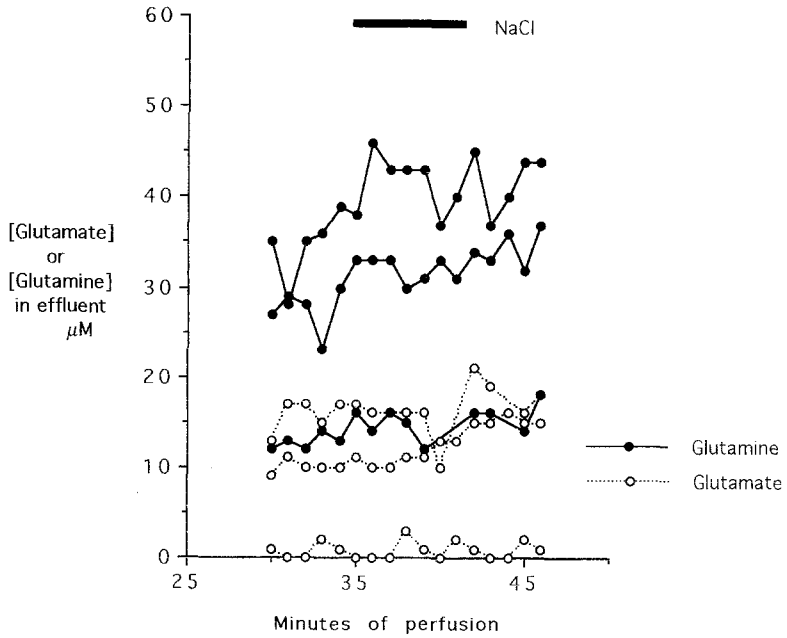

Fig. 3. Three isolated liver perfusions carried out under conditions described in Fig. 1. With the addition of sodium chloride (30 mM, NaCl)

hydroxybutyrate] was >5 mM. This strongly resembles the effect of infusing benzoate (Haussinger et al., 1989), and probably represents an organic acid counter-transport system. No simultaneous changes in glutamine synthesis occurred (over this time period).

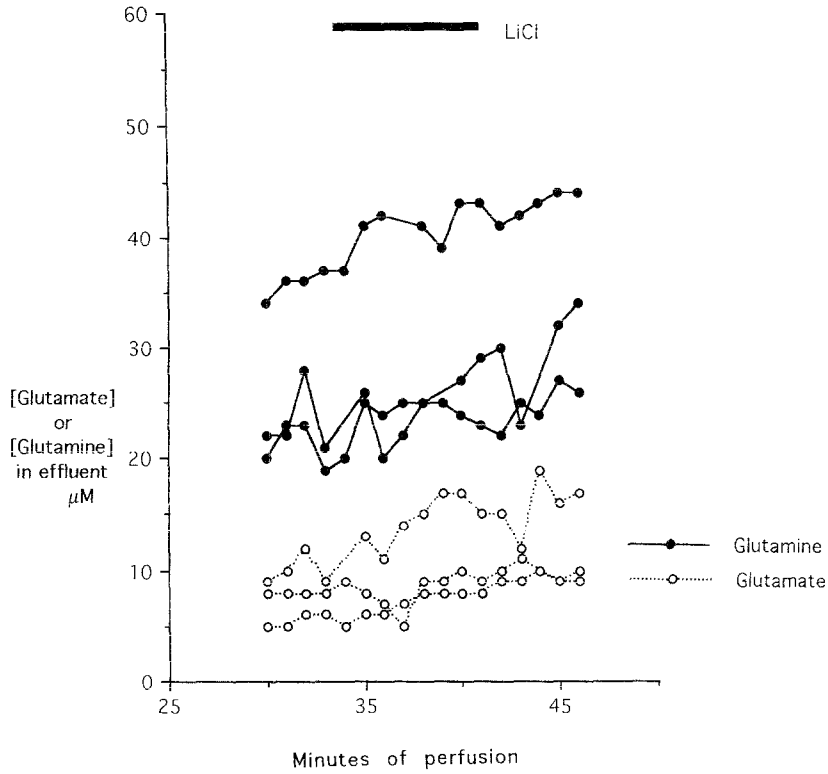

Fig. 4. Three isolated perfusions carried out under conditions described in Fig. 1. With the addition of lithium chloride (Li Cl, 15 mM)

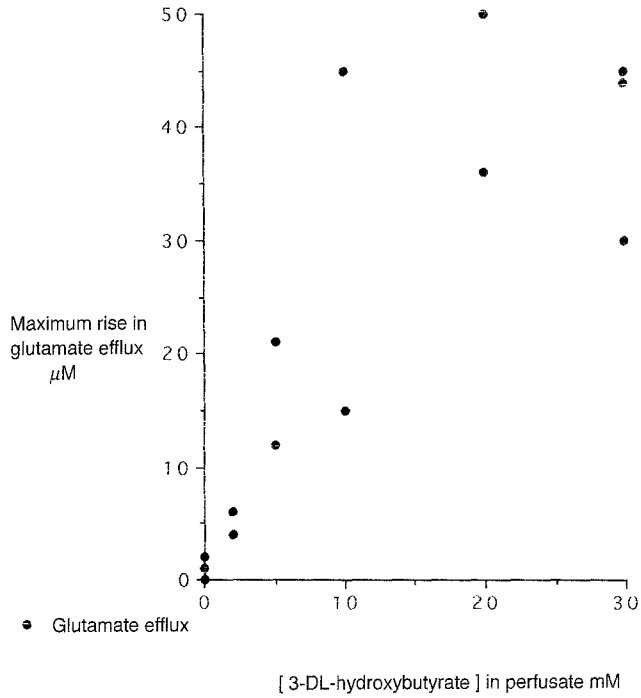

Fig. 5. Maximal rise in glutamate efflux (μM) obtained by perfusing separate livers with: Ammonium 0.6 mM, lactate 2 mM, ornithine 0.1 mM and pyruvate 0.3 mM at pH 7.4. 3-DL-hydroxybutyrate at different concentrations was added to the perfusate for 7 mins after a 30 min equilibration period. Maximal rise in efflux was calculated from the peak efflux during addition of hydroxybutyrate minus the mean efflux prior to the addition

Several (and non mutually exclusive) reasons may account for this change in glutamate export without change in glutamine export; (a) it is possible that the loss of glutamate from the liver *including* the perivenous cells was insufficient to lower intracellular concentrations to a level at which glutamine synthesis and export was compromised (b) that glutamate export was not increased from the perivenous cells which may lack the proposed counter-transporter identified by Haussinger et al., 1989 which was inhibited by quinidine (c) although glutamine synthesis is known to be dependant upon lactate provision (Almond et al., 1991) its synthesis was not inhibited by either 3-hydroxybutyrate or by acetoacetate which have been demonstrated to inhibit the hepatocyte plasma membrane lactate transporter in both isolated hepatocytes and in perfused liver (Metcalf et al., 1986), there is therefore no evidence from this data that the lactate transporter is involved in entry of lactate into perivenous hepatocytes for glutamine synthesis. Under these circumstances lactate presumably enters the hepatocyte by the alternative pathway, non-ionic diffusion (Cohen et al., 1982).

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