Volume 221, number 2, 370–374 FEB 05116 September 1987

Perivenous localisation of Na-dependent glutamate transport in perfused rat liver

P.M. Taylor and M.J. Rennie

Department of Physiology, University of Dundee, Dundee DD1 4HN, Scotland

Received 3 August 1987

Periportal and perivenous hepatocytes differ in their metabolism of blood glutamate (Glu). Uncertainty about the mechanisms of Glu blood-liver exchange led us to characterise, by paired-tracer dilution, a sodium-dependent dicarboxylate transporter (resembling system X^-ag) in sinusoidal membranes of perfused rat liver (V_{max} = 0.18 μmol Glu/g per min, K_m = 0.29 mM Glu). Tracer Glu transport was depressed 65% after necrosis of perivenous hepatocytes by acute CCl_4 treatment, indicating that X^-ag transporter activity is located mainly in these cells, the sites of glutamine (Gln) synthesis from glutamate and ammonia. Modulation of Glu transport may influence the extent of hepatic Gln release.

Glutamine release; Glutamate transport; (Perivenous hepatocyte, Perfused liver)

1. INTRODUCTION

Glu and Gln metabolism in liver is spatially heterogeneous [1–3]. Periportal cells are the predominant site of glutaminase whereas glutamine synthetase is localised 'downstream' in perivenous hepatocytes, where it scavenges ammonia (K_m about 0.1 mM [2]) escaping the urea cycle. Portal Glu is thus involved both in ammonia scavenging and in regulation of plasma Gln [1].

Glu transport in hepatocytes is well characterised, two Na-dependent Glu transporters (glucocorticoid-inducible) having been identified [4,5]. Hepatocyte studies are incapable of distinguishing between either sinusoidal and bile-canalicular membrane transport, or relative periportal/perivenous contributions. The existence or not of Glu transport in sinusoidal membrane vesicles is disputed [6,7], although in intact liver saturable uptake [8] and utilization [1] of portal Glu are clearly demonstrable. Little quantitative information exists on Glu transport in whole liver however, thus precluding direct assessment of possible transporter modulation of Glu flux through intra- and intercellular [2] metabolic pathways. We have therefore investigated Glu transport in the sinusoidal membrane of perfused rat liver by use of a paired-tracer isotope-dilution technique [9].

Correspondence address: P.M. Taylor, Department of Physiology, University of Dundee, Dundee DD1 4HN, Scotland

Abbreviations: HPV, hepatic portal vein; HV, hepatic vein; NMDG, *N*-methyl-D-glucamine; *PS*, permeability-surface area product; UFE, unidirectional fractional extraction; U_{max}, maximal UFE

2. MATERIALS AND METHODS

12-h fasted female Wistar rats (150–180 g body wt) were used. Selective perivenous liver cell necrosis was induced as required by administration of CCl_4 (2.5 ml/kg, 1:1 in olive oil [1]) via stomach tube 20 h before study. Livers of anaesthetised (60 mg/kg nembutal) rats were perfused in situ via the hepatic portal vein (HPV) [10]. The perfusate (Krebs-Henseleit buffer, pH 7.4, 37°C, gassed with 95% O_2/5% CO_2, containing 1% BSA (Miles fraction V) and 15% rejuvenated human red blood cells [11]) was pumped without

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

recirculation at a physiological flow rate [12] of 1.2 ± 0.1 ml/g liver per min ($\dot{Q}$).

Unidirectional amino acid transport was measured by a rapid paired-tracer dilution technique [9]. ^{3}H-labelled amino acid and ^{14}C-labelled sucrose (extracellular marker: [13]) in 0.1 ml perfusate (0.2 MBq total radioactivity: ^{3}H/^{14}C dmp ratio of 5:1) were injected as a bolus into HPV. HV effluent was collected over 2 min (0.2-ml aliquots into 0.2 ml 10% PCA). ^{3}H-tracer uptake by red cells was negligible over this period. Denatured perfusate protein was spun down and 0.12 ml supernatant was processed for dual-channel liquid scintillation counting (Beckman LS 1800) with automatic quench correction. Extracellular ^{14}C-tracer recovery exceeded 95%. Isotope dilution profiles of the tracers (fig.1a) yielded the time course of amino acid UFE; for each sample = 1 − (% ^{3}H dose recovered/% ^{14}C dose recovered). *PS* of the sinusoidal barrier was estimated from U_{max} as $\dot{Q} \times -\ln(1 - U_{max})$ [14]. Unidirectional transport (J) was calculated as *PS* × perfusate [amino acid] and net hepatic amino acid flux as $\dot{Q} \times$ ([amino acid]$_{in}$ − [amino acid]$_{out}$), where [amino acid] represents the amino acid concentration. The kinetics of Glu transport were characterised by measuring tracer amino acid influx in perfusates containing 0.02–20 mM unlabelled amino acid presented in random order. Tracer influx was measured over the final 2 min of 10 min exposure to each perfusate. V_{max} and K_m values were obtained using iterative programs [15] on an Apple IIe microcomputer.

Amino acid concentrations of deproteinised (20% sulphosalicylic acid) samples were measured by either automated amino acid analyser or standard enzymatic methods [16]. Glutaminase and glutamine synthetase activity were measured in homogenised liver (100 mM KCl, 50 mM Tris-HCl buffer, pH 7.8), glutaminase as the rate of Glu production from 20 mM glutamine (pH 8.0, ± 10 mM phosphate [17]) and glutamine synthetase as the rate of [^{3}H]Gln synthesis from [^{3}H]Glu (20 mM Glu, pH 7.4 [18]; Glu and Gln were separated by paper chromatography in 4:1 phenol-water).

Results are presented as means ± 1 SE. Statistical significances were assessed by Student's *t*-test.

3. RESULTS

The mean U_{max} of tracer [^{3}H]Glu, normally 0.44 (e.g. fig.1b), fell to 0.06 in the presence of 20 mM unlabelled Glu and the hepatic *PS* product decreased with increasing perfusate Glu concentra-

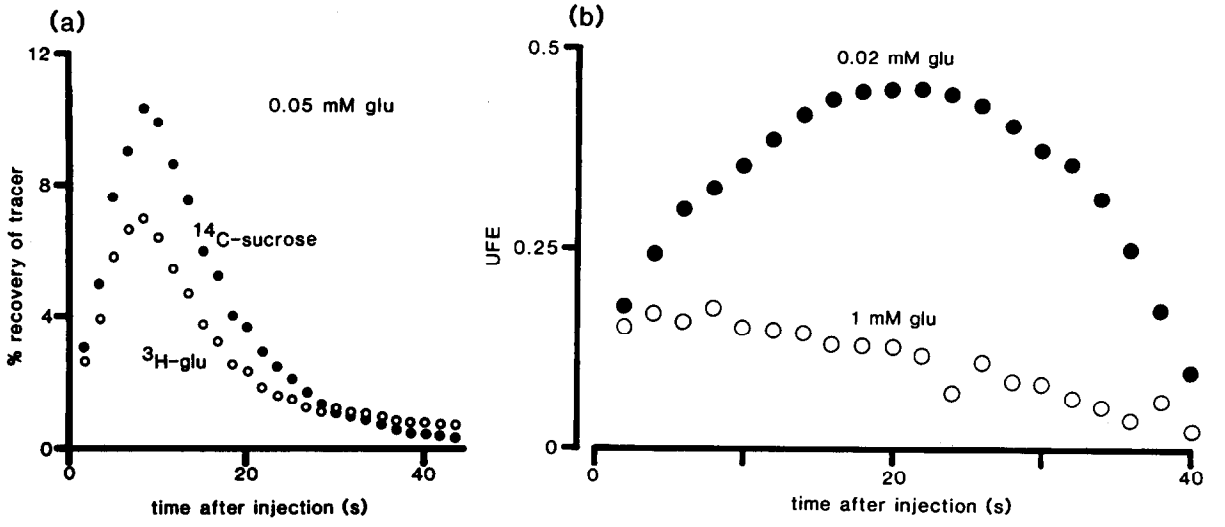

Fig.1. (a) Isotope dilution profiles in effluent from perfused liver after injection of [^{3}H]glutamate + [^{14}C]sucrose mixture into the portal circulation at 0.05 mM perfusate Glu. (b) Liver fractional extraction (UFE) of [^{3}H]glutamate. Tracer uptake is depressed by increased perfusate amino acid concentrations.

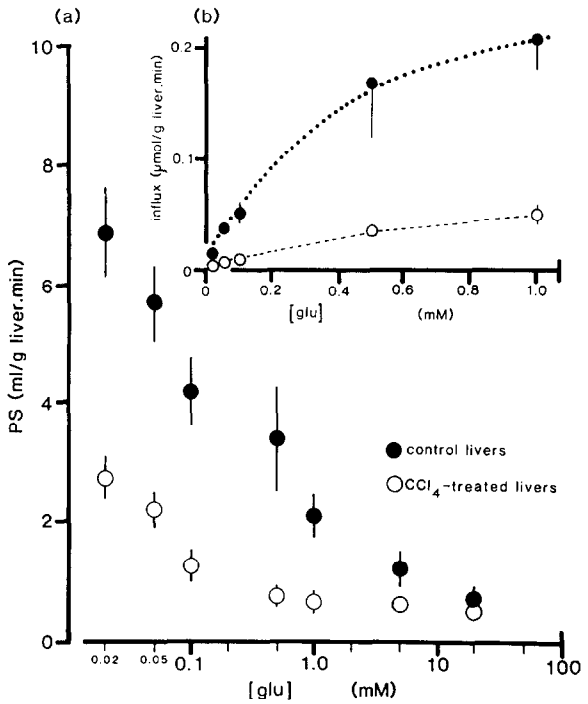

Fig.2. (a) *PS* product and (b) influx of glutamate in perfused liver as a function of perfusate Glu concentration. (●) Control livers, (○) CCl_4-treated livers (n = 4–6).

tion (fig.2a). Thus movement of Glu from blood to hepatocytes included a saturable mechanism. Glu influx considerably exceeded net hepatic flux, which was −0.05 ± 0.01 and +0.07 ± 0.02 μmol/g per min at perfusate Glu of 0.02 and 0.5 mM, respectively. In post-absorptive rats in vivo HPV plasma Glu was 0.075 ± 0.011 mM (n = 7). Co-injection of 50 mM unlabelled L-Glu, D-Glu, L-Asp, D-Asp and L-Gln with [^{3}H]Glu tracer depressed the initial tracer extraction by a maximum of 85 ± 4, 8 ± 7, 49 ± 12, 61 ± 9 and 4 ± 3%, respectively (n = 3–4). Flux-concentration data were fitted to a first-order Michaelis-Menten function (J vs [Glu] to a hyperbola; PS vs ln[Glu] to an inverse sigmoid curve [19]), and kinetic characteristics were obtained for saturable Glu transport (V_{max} = 0.18 ± 0.03 μmol/g per min, K_m = 0.29 ± 0.11 mM).

Glu influx was markedly depressed in CCl_4-treated rats, tracer [^{3}H]Glu extraction being reduced to 0.17 and influx to about 35% of normal over 0.02–0.5 mM Glu (fig.2). CCl_4-treated rat liver showed a 67% depletion of hepatic glutamine synthetase but no loss of glutaminase activity (table 1), results consistent with selective necrosis of perivenous hepatocytes. Glutamine transport was unaffected by the treatment (mean unidirectional [^{3}H]Gln extractions of 0.73 in both control and CCl_4-treated livers at 0.5 mM Gln [20]), indicating a specific loss of perivenous Glu-transport capacity.

Table 1

Glutaminase and glutamine synthetase activities in control livers and in those from rats treated with CCl_4 (2.5 ml/kg)

Enzyme	Enzyme activity in liver homogenate (μmol/g per min: n = 4–7)	
	Control	CCl_4-treated
Glutaminase	5.78 ± 0.73	4.94 ± 0.56
Glutamine synthetase	7.6 ± 1.5	2.5 ± 0.45[a]

[a] Significantly different from control value, $p < 0.01$

About 60% of glutaminase activity was phosphate-dependent

Limited substitution of perfusate Na^+ (replacement of 118 mM NaCl by choline or NMDG chlorides, with retention of 25 mM $NaHCO_3$) significantly reduced hepatic Glu and Gln influx (table 2). Ammonia (0.8 mM in perfusate) was without effect on Glu transport at 0.05 mM perfusate Glu (not shown).

4. DISCUSSION

We have observed extractions of tracer Glu and Gln from portal perfusate during a single passage through liver sinusoids as high as those found previously in rat liver in vivo [21]. The results indicate that hepatocytes (which constitute 83% of liver mass [22]) in vivo extract Glu from the HPV by a low capacity, 'high-affinity' carrier-mediated process. L-Glu, L-Asp and D-Asp were effective inhibitors of [^{3}H]Glu influx and, although competitive and non-competitive inhibition were not differentiated, previous studies show that these amino acids share a dicarboxylate transporter designated system X^-ag [4,7]. At physiological

Table 2

The effects of extracellular sodium replacement with choline or NMDG on glutamate and glutamine transport in perfused rat liver

Amino acid	Amino acid influx (μmol/g per min: n = 4–5)		
	Control	Choline-Krebs	NMDG-Krebs
Glutamate	0.028 ± 0.003	0.008 ± 0.002[b]	0.010 ± 0.003[b]
Glutamine	0.55 ± 0.06	0.20 ± 0.02[b]	0.34 ± 0.05[a]

Values significantly different from control, [a] $p < 0.05$; [b] $p < 0.01$

amino acids share a dicarboxylate transporter designated system X^-ag [4,7]. At physiological HPV plasma Glu, approx. 66% of Glu influx is into the small (7% by vol. [3]) population of perivenous hepatocytes, i.e. transport may be 25-times faster into perivenous cells than into periportal cells. The capacity of system X^-ag is an order of magnitude lower than that of Na-dependent systems transporting Ala and Gln in perfused rat liver [12], but physiologically it will still extract about 34% of portal Glu during a single passage (0.075 mM Glu, 1.2 ml/g per min portal flow [12]). Residual Glu extraction in CCl_4-treated rat liver is low, indicating that periportal Glu exchange may be predominantly diffusional.

HPV [^{14}C]Glu is maximally utilized by perfused rat liver (yielding $^{14}CO_2$) at 0.2 μmol/g per min with a K_m of about 0.15 mM [1]; these values are similar to the observed transport characteristics, indicating rapid turnover of the perivenous Glu pool. The good match between Glu entry and metabolism indicates that (i) little or no portal Glu is transported into the bile and (ii) there is likely to be no significant Glu efflux across perivenous sinusoidal membranes.

Glu is simultaneously taken up and released by perfused liver [1]. Most Glu uptake is by perivenous cells which release no Glu, thus Glu exchange (leading to net efflux) must occur across blood-facing membranes of periportal tissue. Periportal Glu accumulation is evident when mitochondrial glutaminase activity is stimulated in perfused liver [1], and one barrier to Glu export is likely to be the sinusoidal membrane. In this study hepatic Glu balance becomes positive as portal delivery increases due to a relative increase in perivenous extraction over periportal efflux.

Glu and Asp have similar transport characteristics by system X^-ag [7]. Surface membrane transport may be important for control of the intracellular concentrations of Glu and Asp, and possibly for regulating interaction between cytosol and mitochondria (e.g. [2]). Na-dependent transport permits cytosolic Glu to be maintained above the plasma concentration [5]. It has been argued that neither ammonia nor Glu availability exerts appreciable physiological control over perivenous Gln synthesis [2], although about 60% of transported Glu is available to glutamine synthetase. Nevertheless, the need for close coordination of Glu transport and Gln synthesis appears to be exhibited by the finding that Glu transporters are induced in parallel with Gln synthetase by glucocorticoids in cultured hepatocytes [5,23]; thus Glu availability by transport may indeed exert some control over Gln synthesis.

The present results help explain apparently conflicting reports on the localisation of Glu transport in liver plasma membrane (e.g. [5–7]). There appear to be two Na-dependent transport systems on the hepatocyte membrane [4,5]: one is located on the bile-canalicular surface and has high substrate affinity (K_m about 10–25 μM); the second, characterised here, is on the perivenous sinusoidal membrane and has slightly lower affinity (K_m = 0.29 mM). Masking of Glu transport activity in perivenous sinusoidal membranes by the larger pool of periportal membranes in hepatocytes and vesicle preparations may partly explain why the latter system has been overlooked in some previous studies (e.g. [6]).

ACKNOWLEDGEMENTS

This work was supported by The Wellcome Trust, Medical Research Council, British Diabetic Association and the University of Dundee. We thank C. Kuret and C.J. Egan for assistance with some perfusion experiments, and S. O'Rourke and B. Weryk for amino acid analysis.

REFERENCES

[1] Häussinger, D. and Gerok, W. (1983) Eur. J. Biochem. 136, 421–425.
[2] Sies, H. and Häussinger, D. (1984) in: Glutamine Metabolism in Mammalian Tissues (Häussinger, D. and Sies, H. eds) pp.78–97, Springer, Heidelberg.
[3] Gebhardt, R. and Mecke, D. (1983) EMBO J. 2, 567–570.
[4] Gazzola, G.C., Dall'Asta, V., Bussolati, O., Makowske, M. and Christensen, H.N. (1981) J. Biol. Chem. 256, 6054–6059.
[5] Gebhardt, R. and Mecke, D. (1983) FEBS Lett. 161, 275–278.
[6] Ballatori, N., Moseley, R.H. and Boyer, J.L. (1986) J. Biol. Chem. 261, 6216–6221.
[7] Sips, H.J., DeGraaf, P.A. and Van Dam, K. (1982) Eur. J. Biochem. 122, 259–264.
[8] Pardridge, W.M. and Jefferson, L.S. (1975) Am. J. Physiol. 228, 1155–1161.
[9] Yudilevich, D.L. and Mann, G.E. (1982) Fed. Proc. 41, 3045–3053.
[10] Hems, R., Ross, B.D., Berry, M.N. and Krebs, H.A. (1966) Biochem. J. 101, 284–292.
[11] Valeri, C.R. (1974) in: The Red Blood Cell, 2nd edn (Surgenor, D.M. ed.) pp.528–532, Academic Press, New York.
[12] Taylor, P.M. and Rennie, M.J. (1986) Biochem. Soc. Trans. 14, 1069–1070.
[13] Bravo, I.R. and Yudilevich, D.L. (1971) Am. J. Physiol. 221, 1449–1455.
[14] Renkin, E.M. (1959) Am. J. Physiol. 197, 1205–1210.
[15] Barlow, E. (1982) Biodata Handling with Microcomputers, Elsevier, Amsterdam, New York.
[16] Bergmeyer, H.U. (1974) Methods of Enzymatic Analysis, Springer, Berlin.
[17] Huang, Y.-Z. and Knox, W.E. (1976) Enzyme 21, 408–426.
[18] Lund, P. (1970) Biochem. J. 118, 35–39.
[19] Bassingthwaighte, J.B. (1982) Fed. Proc. 41, 3031–3032.
[20] Rennie, M.J. and Taylor, P.M. (1987) J. Physiol. 387, 94P.
[21] Pardridge, W.M. (1977) Am. J. Physiol. 232, E492–E496.
[22] Wiebel, E.R., Staubli, W., Gnagi, H.R. and Hess, F.A. (1969) J. Cell Biol. 42, 68–91.
[23] Gebhardt, R., Kleemann, E. and Mecke, D. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1243–1244.