Differential effects of starvation on alanine and glutamine transport in isolated rat hepatocytes

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At physiological concentrations, alanine transport in hepatocytes from starved rats is faster than in hepatocytes from fed rats. The degree of increase is much less than previously reported for 2-aminoisobutyrate in the same concentration range. Glutamine transport is not stimulated on starvation. This provides evidence that the transport systems for alanine and glutamine in isolated hepatocytes are controlled separately.

The rate of transport of the amino acid analogue 2-aminoisobutyrate in hepatocytes from starved rats is increased compared with the corresponding rate in hepatocytes from fed rats (Fehlmann *et al.*, 1979). This increase in transport activity has been attributed to the emergence on starvation of a transport system with a low K_m for 2-aminoisobutyrate. Similar low- K_m systems for 2-aminoisobutyrate have been reported in hepatocytes from partially hepatectomized rats (Le Cam *et al.*, 1979) and from neonatal-rat liver (Bellemann, 1981).

The effect of starvation on the transport of neutral amino acids into hepatocytes has not been systematically investigated. From previous work in this laboratory on transport in hepatocytes from fed (McGivan *et al.*, 1981) and starved (Joseph *et al.*, 1978) rats, it appeared that the overall rate of alanine transport was somewhat increased as a result of starvation. It has subsequently been shown that the transport of alanine into hepatocytes can limit alanine transport under certain conditions (Sips *et al.*, 1980; McGivan *et al.*, 1981), so it is important that the effect of starvation on alanine transport should be quantified. In hepatocytes, unlike several other cell types, glutamine is transported by a system separate from that which catalyses alanine transport. This transport system has been identified and characterized by Kilberg *et al.* (1980), who also reported that glutamine transport was subject to adaptive changes on starvation parallel to those shown by alanine transport.

The major object of the present paper is to define the effect of starvation on the overall transport of alanine and glutamine in hepatocytes suspended in near-physiological media. We have not attempted to resolve this transport into component systems determined by artificial amino acid analogues.

Materials and methods

Hepatocytes were isolated as described previously (Joseph *et al.*, 1978) from the livers of 300 g male Wistar rats, either fed normally or starved for 48 h. Unless otherwise stated, cells were incubated in Krebs–Henseleit bicarbonate buffer (pH 7.4), containing 2% (w/v) defatted bovine serum albumin. Control experiments showed that the addition of sodium oleate to this medium did not increase the rate of alanine transport. Viability of cells was tested with Trypan Blue, and only those preparations with greater than 90% viability were used in experiments.

Transport of alanine and glutamine was determined as described previously (Joseph *et al.*, 1978; McGivan, 1979), by using silicone-layer centrifugation to terminate the transport process. Amino-oxyacetate (1 mM) was present in all incubations to inhibit alanine metabolism (Edmondson *et al.*, 1977; Joseph *et al.*, 1978). It has been shown previously that there is no net metabolism at concentrations less than 2 mM over a period of 20 min (Lund & Watford, 1976; Joseph *et al.*, 1978). In experiments with perfused liver, Haussinger & Sies (1979) observed a release of $^{14}\text{CO}_2$ from [U- ^{14}C]-glutamine in the absence of net glutamine metabolism. This was attributed to futile cycling. Although it is not possible to exclude some loss of $^{14}\text{CO}_2$ from labelled glutamine in the present experiments, it is unlikely that such loss of label would be significant during the measurement of initial rates. Exchange of ^{14}C label between glutamine and oxoglutarate would be expected to be inhibited to a large extent in the presence of amino-oxyacetate, which inhibits aspartate aminotransferase.

Alanine was assayed enzymically (Williamson *et al.*, 1967), and cell protein was determined by a biuret method (Gornall *et al.*, 1949).

Results

Fig. 1 shows the time course of the uptake of 1 mM-alanine and 1 mM-glutamine in hepatocytes isolated from one fed and one starved rat in a representative experiment. The initial rate of alanine transport increased on starvation, whereas that of glutamine remained unchanged.

The concentration-dependence of the effect is shown in Table 1. A significant increase in the rate of alanine transport was observed between fed and starved rats at all concentrations of alanine studied from 0.25 to 10 mM. There was no significant difference in the rate of glutamine transport between fed and starved rats over the same concentration range.

Table 2 shows the effect of starvation on glut-

Fig. 1. Time course of the uptake of alanine (a) and glutamine (b) in hepatocytes from fed (●) and starved (○) rats. The uptake of 1 mM-alanine and 1 mM-glutamine at 37°C was measured radioisotopically. The results from hepatocytes from one fed and one starved rat are shown and the values are means of duplicate determinations. No metabolism of either alanine or glutamine occurred under these conditions during the first 10 min. At 15 min, the alanine uptake measured by enzyme assay was about 20% less than that measured radioisotopically.

Table 1. Effect of starvation on the overall initial rates of transport of glutamine and alanine in hepatocytes suspended in Krebs–Henseleit medium

Cells were prepared from fed and 48 h-starved rats as described in the Materials and methods section. The initial rate of uptake at 37°C was measured after 2 min. The values shown are the overall means $\pm$ S.E.M. for the numbers of separate cell preparations shown in parentheses. Each rate determination was performed in duplicate. The significance of the difference in rates between hepatocytes from fed and starved rats was assessed by Student's *t* test: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Amino acid concn. (mM)	Initial rate of amino acid uptake (nmol/min per mg)			
	Alanine		Glutamine	
	Fed	Starved	Fed	Starved
0.25	0.55 $\pm$ 0.04 (4)	1.04 $\pm$ 0.07 (5)***	1.63 $\pm$ 0.15 (4)	1.66 $\pm$ 0.18 (4)
0.5	1.03 $\pm$ 0.05 (4)	1.82 $\pm$ 0.10 (5)***	2.54 $\pm$ 0.28 (4)	3.05 $\pm$ 0.33 (4)
1	1.90 $\pm$ 0.11 (4)	3.44 $\pm$ 0.13 (5)***	4.40 $\pm$ 0.51 (4)	4.88 $\pm$ 0.51 (4)
2	3.82 $\pm$ 0.18 (4)	5.33 $\pm$ 0.25 (5)**	6.86 $\pm$ 1.06 (4)	6.98 $\pm$ 0.55 (4)
4	5.91 $\pm$ 0.99 (3)	9.29 $\pm$ 0.76 (5)*	9.01 $\pm$ 1.07 (4)	9.23 $\pm$ 1.12 (3)
6	7.07 $\pm$ 0.78 (4)	11.67 $\pm$ 0.73 (5)**	9.35 $\pm$ 0.76 (4)	10.10 $\pm$ 0.80 (4)
10	10.14 $\pm$ 0.81 (4)	14.82 $\pm$ 1.27 (4)*	10.43 $\pm$ 0.37 (3)	10.07 $\pm$ 0.47 (4)

Table 2. *Alanine and glutamine transport in Na⁺-free medium*

Cells were washed and suspended in a modification of Krebs–Henseleit medium in which the NaCl and NaHCO₃ were replaced by corresponding concentrations of choline chloride and choline bicarbonate respectively. Other details are as in the legend to Table 1.

Amino acid concn. (mM)	Initial rate of amino acid uptake (nmol/min per mg)			
	Alanine		Glutamine	
	Fed	Starved	Fed	Starved
0.25	0.05 ± 0.003 (3)	0.09 ± 0.02 (4)	0.27 ± 0.03 (4)	0.40 ± 0.02 (4)*
0.5	0.09 ± 0.02 (6)	0.14 ± 0.01 (6)*	0.51 ± 0.05 (7)	0.66 ± 0.11 (6)
1	0.16 ± 0.02 (4)	0.32 ± 0.03 (4)**	1.07 ± 0.17 (5)	1.08 ± 0.10 (4)
2	0.28 ± 0.03 (6)	0.52 ± 0.03 (6)***	1.69 ± 0.19 (6)	1.69 ± 0.13 (6)
4	0.52 ± 0.05 (4)	1.01 ± 0.18 (4)*	2.28 ± 0.26 (6)	2.58 ± 0.32 (4)
6	0.76 ± 0.06 (3)	1.22 ± 0.28 (3)	2.93 ± 0.33 (5)	3.37 ± 0.38 (4)
10	0.96 ± 0.13 (4)	1.51 ± 0.19 (4)	3.98 ± 0.30 (4)	3.48 ± 0.73 (3)

amine and alanine transport when Na⁺ in the medium was replaced with choline. There was a significant increase in the Na⁺-independent rate of transport of alanine, but not that of glutamine. Nevertheless, these results, taken together with those in Table 1, show that 90% of the increase in the overall rate of alanine transport was Na⁺-dependent.

The transport of glutamine in the choline medium was much faster than that of alanine and was also saturable at high concentrations of glutamine. One interpretation of these results is that the glutamine-transport system, but not the alanine-transport system, is able to use choline in place of Na⁺ to some extent. The glutamine-transport system is known to have a relatively high tolerance for replacement of Na⁺ by Li⁺ (Kilberg *et al.*, 1980).

In several experiments, alanine transport was also measured by enzymic analysis of alanine in the pellet and in the supernatant. It was consistently found that, in cells from both fed and starved rats, the uptake of alanine measured radioisotopically was identical with that measured by enzymic assay over the first 10 min. No alanine metabolism occurred during this period, although there was some metabolism of alanine between 10 and 20 min.

The possibility that the different apparent rates of alanine transport measured in cells from fed and starved rats could be influenced by different concentrations of endogenous alanine in the cells was considered. It was found that hepatocytes contained 0.5–1 nmol of endogenous alanine/mg of protein when suspended in an alanine-free medium. There was no significant difference between the endogenous contents of alanine in cells from fed and starved rats (but see Sips *et al.*, 1980). In control experiments, the intracellular content of alanine was varied by preincubating hepatocytes with alanine at concentrations of between 0 and 0.25 mM for 20 min in the presence of amino-oxyacetate. This procedure

had no significant effect on the initial rate of uptake of 2 mM-alanine added at the end of this period. It was concluded therefore that the increase in the rate of alanine transport on starvation was not an artefact of the experimental procedure used.

Discussion

The results presented in this paper have two major implications. Firstly, alanine transport in isolated hepatocytes is increased as a result of starvation. The increase is nearly all Na⁺-dependent and is presumably the result of increased activity of one or more Na⁺-dependent transport systems. At physiological concentrations of alanine (below 1 mM) and Na⁺ ions, alanine transport was increased by 80–90%. At higher concentrations of alanine, the increase was less marked. These results are consistent with the previous finding of Kilberg *et al.* (1979) that the overall transport of 0.1 mM-alanine was increased by 60% in hepatocytes from starved as compared with fed rats.

Studies on the transport of 2-aminoisobutyrate in hepatocytes have shown that the initial rate of transport of 0.1 mM-2-aminoisobutyrate was increased some 20-fold on starvation of the donor animals (Fehlmann *et al.*, 1979). The rate of transport of 50 mM-2-aminoisobutyrate was increased by only 50%. It was shown that a system with a low *K_m* for 2-aminoisobutyrate developed on starvation. The results presented here show that alanine transport is induced to a very much lower extent than is the uptake of 2-aminoisobutyrate at comparable concentrations. The percentage increase in the transport of 1 mM-alanine is comparable with that observed for 50 mM-2-aminoisobutyrate. The uptake of 2-aminoisobutyrate (Le Cam & Freychet, 1977) in hepatocytes is much slower than that of alanine (Edmondson *et al.*, 1977; Joseph *et al.*,

1978; McGivan *et al.*, 1981) and the apparent K_m for Na^+ -dependent 2-aminoisobutyrate transport is 5 times that for alanine. It is clear from the present results that observations on 2-aminoisobutyrate transport in hepatocytes cannot be directly extrapolated to predict quantitative changes in the transport of alanine. In particular, the very large increase in the rate of transport of 2-aminoisobutyrate on starvation is not reflected in similarly large increases in alanine transport at physiological alanine concentrations.

The results in the present paper show that glutamine transport is not increased in hepatocytes as a result of starvation of the donor animal. This finding, together with the observation that replacement of Na^+ by choline has a much greater effect on the transport of alanine than on that of glutamine, substantiates the existence of separate carrier systems for glutamine and alanine (Kilberg *et al.*, 1980) and indicates that these systems are separately controlled. Kilberg *et al.* (1980) observed a doubling in the rate of glutamine transport on culturing hepatocytes for 8 h in the absence of amino acids. Under the same conditions the transport of 2-(methylamino)isobutyric acid increased 16-fold. These findings on cultured hepatocytes cannot be compared directly with the present results on freshly prepared hepatocytes in suspension.

In hepatocytes suspended in Krebs-Henseleit bicarbonate buffer, alanine transport is inducible on starvation of the donor animal, whereas glutamine transport is not. These results are of interest in the light of reports that alanine transport is limiting for alanine metabolism in hepatocytes. Sips *et al.* (1980) concluded that alanine transport was limiting for metabolism at physiological concentrations of alanine in both fed and starved rats. McGivan *et al.* (1981), studying only fed rats, concluded that alanine transport was not limiting at alanine concentrations above 1 mM, but was limiting at physio-

logical alanine concentrations. In contrast, the metabolism of glutamine in hepatocytes is limited by the enzyme glutaminase and not by glutamine transport (Joseph & McGivan, 1978).

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