Glutamate uptake by cultured rat hepatocytes is mediated by hormonally inducible, sodium-dependent transport systems

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Glutamate uptake by rat hepatocytes in primary monolayer culture was found to be mediated by a Na^+-independent and by two Na^+-dependent transport systems of high and low affinity. Inhibition studies with cysteate and other model amino acids rules out the participation of the neutral amino acid transport systems A, ASC, and N and revealed that the Na^+-dependent agencies represent unequivocally anionic transport systems. Na^+-dependent uptake of glutamate in isolated hepatocytes was slow compared to the Na^+-independent portion, but increased spontaneously during cultivation. In the presence of dexamethasone it was stimulated about 10-fold at the second day of cultivation.

Cell culture *Dexamethasone* *Glutamate transport* *Hepatocyte*
Hormonal control *Na^+-dependence*

1. INTRODUCTION

The recent discovery of a strictly pericentral localization of hepatic glutamine synthetase (EC 6.3.1.1) has shed new light on the liver as a potentially powerful site for glutamine synthesis and export [1]. Although only few experimental conditions characterized by net glutamine synthesis have been described so far (cf. [2]), the simultaneous requirement for glutamine utilization within the main part of liver parenchyma [1] makes a compensatory high glutamine synthesis indispensable for maintaining blood glutamine concentrations irrespective of whether there is a netproduction or consumption of this amino acid. This aspect has prompted us to investigate what substrates might be important precursors for glutamine synthesized by liver parenchymal cells.

Extracellular glutamate would be an ideal precursor which, if taken up in sufficient amounts, could directly participate in the glutamine synthetase reaction. However, since the studies of Hems et al. in 1968 [3] using liver slices, it is a widely held view that glutamate is poorly taken up by liver parenchymal cells. Accordingly, the high intracellular concentration of glutamate has been considered as the expression of a strong permeability barrier and as the result of a high intracellular production of glutamate from various precursors.

Despite this apparently convincing evidence, however, observations on the utilization of glutamate by the liver in different physiological states have been reported sporadically [4,5]. Recently, the introduction of primary monolayer cultures has facilitated and promoted the investigation of amino acid transport by hepatocytes [5–9] and particularly of the uptake of anionic amino acids [10–13]. Here, we used this experimental model to investigate glutamate transport and its possible hormonal regulation.

2. MATERIALS AND METHODS

Isolation of liver parenchymal cells from male Sprague–Dawley rats (220–290 g) fed ad libitum and details of cultivation were described in [14,15]. Differing from the protocol described in [15] cultures were maintained in serum-free W/AB 77 medium after an initial period of 3 h and no hor-

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mones were present throughout cultivation except otherwise stated.

The medium used for uptake measurements was either sodium-containing Krebs-Ringer bicarbonate buffer (pH 7.4) or a buffer of the same composition in which choline bicarbonate or choline chloride were substituted for the corresponding sodium-salts. Both media contained 0.2 μCi/ml L-[U-^{14}C]glutamate (280 mCi/mmol; Amersham-Buchler, Braunschweig) and were adjusted with unlabelled glutamate to a final concentration of 0.5 mM. For determination of kinetic constants L-glutamate concentration was varied between 0.01 mM and 20 mM and data were analyzed using a computer fitting program.

Glutamate transport into cultured hepatocytes was measured by adding 2 ml of the uptake medium at 37°C to each dish for the desired interval (usually 5 min) followed by removal of the medium by aspiration and 3 quick washes with ice-cold saline. Finally, 1 ml of suspension buffer [16] was added and aliquots were taken for determination of residual extracellular glutamate before cells were scraped off with a rubber policeman. Homogenates were prepared by sonication [17] and aliquots were taken for determination of radioactivity and of protein as in [18].

3. RESULTS AND DISCUSSION

The uptake of glutamate by hepatocytes in primary culture was found to proceed linear with time over 20 min (fig.1) and to decline only slightly within 1 h. On the basis of these results, a 5-min interval was chosen for measuring the initial velocity of glutamate transport in subsequent experiments.

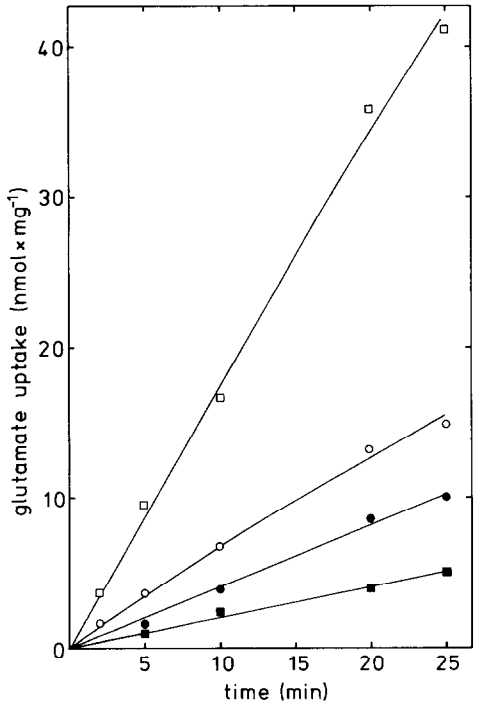

Fig.1. Time course of L-glutamate uptake in cultured hepatocytes. Hepatocytes were maintained in the absence (○, ●) or presence (□, ■) of 10^{-7} M dexamethasone for two days. L-glutamate uptake was measured in sodium ○, □ or choline ●, ■ containing Krebs-Henseleit buffer as described in section 2. The data represent averages of dublicate determinations.

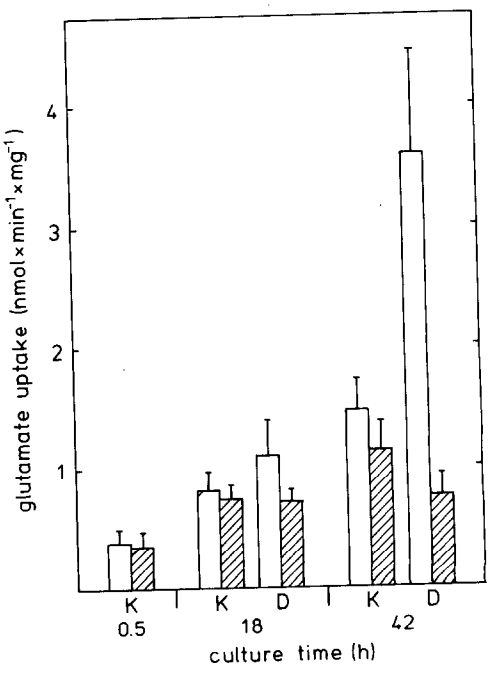

Fig.2. Time course for stimulation of L-glutamate uptake by dexamethasone. Hepatocytes were cultured in the absence (K) or presence (D) of 10^{-7} M dexamethasone. Total (open bars) and Na^+-independent uptake (hatched bars) were determined. The data represent means ± SD calculated from duplicate or triplicate determinations in 3–5 different cultures.

Glutamate uptake by the hepatocytes exhibited saturation kinetics with respect to glutamate (not shown). When measured in Na^+-containing or Na^+-free (choline) medium in the presence of 0.5 mM glutamate a Na^+-dependent portion of the uptake could be detected. In freshly isolated cells the velocity of this transport was relatively slow (0.03 $nmol.min^{-1}.mg^{-1}$) compared to that of the Na^+-independent portion (0.36 $nmol.min^{-1}.mg^{-1}$; cf. fig.2) in agreement with [10]. Whether this is a unique property of isolated hepatocytes or a characteristic of the liver of the fed animal is not known at present.

In the course of cultivation a spontaneous transient increase of the Na^+-dependent transport capacity could be observed (fig.2) culminating between the second and the third day. Careful analysis on the second day of cultivation revealed the existence of 3 different components, one Na^+-independent (app. K_m: 0.53 mM) and two Na^+-dependent systems of high (app. K_m 0.021 mM) and low affinity (app. K_m 3.27 mM), respectively (not shown). This latter finding is in close accord with the recent discrimination in [11] of two Na^+-dependent transport agencies for the model substrate cysteinsulfinate in cultured hepatocytes.

The Na^+-dependent glutamate uptake was further characterized by inhibition studies. Aspartate and cysteate, a model substrate for anionic amino acid transport [19], almost completely blocked the uptake of glutamate (table 1) indicating that glutamate and aspartate share a common route via unequivocally anionic transport systems. Conversely, L-glutamate has been shown to be a potent inhibitor of cysteate uptake in cultured rat hepatocytes [11]. The strong inhibition exerted by cysteate also argues against a significant contribution of the Na^+-dependent transport systems for neutral amino acids which would be restricted to the protonated Zwitterion form of glutamic acid as has been found in the Ehrlich ascites tumor cell [19]. Furthermore, involvement of systems A and N could be ruled out by the failure of the model substrates α-methylaminoisobutyrate [8] and glutamine [7] to inhibit glutamate uptake (table 1). Alanine and serine which are strong substrates of system ASC did not affect glutamate uptake indicating that system ASC is not involved. The partial inhibition exerted by cysteine may be attributed to the fraction of molecules being present in the anionic form at pH 7.4 as in [11].

Table 1

Inhibition of L-glutamate uptake rates in cultured hepatocytes by selected amino acids

Inhibitor tested (20 mM)	% Control	
	Total uptake	Na^+-Dependent uptake
Cysteate	29 ± 3	5 ± 2
Aspartate	43 ± 5	23 ± 4
MeAIB	113 ± 6	106 ± 4
Glutamine	103 ± 10	99 ± 6
Alanine	87 ± 9	95 ± 7
Serine	96 ± 8	101 ± 7
Cysteine	79 ± 12	73 ± 11

Hepatocytes were cultured for 2 days in the presence of 10^{-7} M dexamethasone. Values are expressed as percentages of controls (without inhibitor) and represent means ± SD calculated from duplicate determinations of uptake rates in 3 cultures

Hitherto, system A for neutral amino acid transport was considered unique with respect to hormonal regulation (review [9]). Here, we were able to show that glutamate transport represents a second example of a hormonally inducible transport system. Cultivation in the presence of dexamethasone gave rise to a pronounced induction of glutamate uptake (fig. 1) which proceeded slowly during the first day and was most prominent between the first and the second day of cultivation (fig.2). While total uptake in the presence of 0.5 mM glutamate was more than doubled on the second day, the Na^+-independent portion was simultaneously decreased (fig.1,2). Thus, the Na^+-dependent uptake is stimulated more than 8-fold by dexamethasone. In case of both the high and low affinity system only V_{max} seemed to be increased, whereas no change in K_m could be detected. It should be emphasized that the capacity of the induced glutamate uptake is comparable to that of glutamine transport by system N which is considered one of the most active amino acid transport systems in the liver parenchymal cell [7].

It is interesting to note that the Na^+-dependent systems for glutamate uptake seem to represent the first transport agencies which are directly influenced by dexamethasone, whereas in the case of

system A, glucocorticoids have been shown to exert a permissive action only (for discussion see [9]). The finding of a glucocorticoid-inducible transport of glutamate fits well with the observation in [5] that hepatic glutamate utilization is enhanced by feeding a high protein diet. Additionally, we were able to show that glutamate is efficiently converted into glutamine in dexamethasone-treated hepatocyte cultures (in preparation). The fact that the rate of the stimulated glutamate transport amounts to about 1/4 of the activity of glutamine synthetase determined in vitro [17] suggests that at least under certain conditions glutamine synthesis might be controlled by glutamate uptake.

The previous failure to detect glutamate transport in liver slices [3] is not fully understood. The interesting hormonal regulation of glutamate transport might provide a basis for the understanding of this discrepancy. Another point may be that cultured hepatocytes are superior to liver slices with respect to viability and functional competence. Despite these considerations it remains to be established with what capacity the detected glutamate transport systems are operative in the liver in different physiological states which may differ strikingly in the utilization of extracellular glutamate [5]. Furthermore, in view of the apparent compartmentation of the liver parenchyma with respect to glutamine [1] and urea [21,22] synthesis the question arises whether different glutamate-transporting agencies are partitioned between apparently different parenchymal cells.

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