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ABSENCE OF PREFERENTIAL GLUTAMINE UPTAKE INTO NEURONS — AN INDICATION OF A *NET* TRANSFER OF TCA CONSTITUENTS FROM NERVE ENDINGS TO ASTROCYTES?

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SUMMARY

Uptake kinetics for glutamine were studied in several different neuronal preparations (perikarya prepared by gradient centrifugation, cultured cortical neurons, cultured, presumably glutamatergic cerebellar neurons, and brain prisms). In no case were any indications found of a high affinity uptake but a rather efficient low affinity uptake did occur. A similar, equally efficient low affinity uptake is, however, found in astrocytes. Thus, no preferential glutamine uptake occurs into neurons. It is, therefore, *not* likely that a net flow of glutamine takes place from astrocytes to neurons, compensating for the loss of TCA constituents when glutamate and GABA are released.

Studies of the metabolic fate of different radioactive precursors (e.g., glucose and acetate) have suggested that the mature mammalian brain in vivo contains at least two 'metabolic compartments' which might represent different cell types and/or different subcellular structures (for details and references, see 1 and 7). Most concepts of this 'metabolic compartmentation' envisage a transfer of GABA [31] or glutamate [4,5] from a 'large' presumably neuronal to a 'small', presumably astrocytic compartment as well as a quantitatively corresponding flow of glutamine in the opposite direction, serving the purpose of maintaining the pools of tricarboxylic acid cycle (TCA) intermediates in both compartments. A pronounced release of glutamate and GABA from neurons is well established (for references see, e.g., 13 and 15) and fits, together with a preferential localization of the glutamine synthetase in glial cells [5,18,30] and more specifically in astrocytes [20] into this picture.

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We have previously demonstrated an intense high affinity uptake of glutamate into normal astrocytes in primary cultures [14] as well as a high glutamine synthetase activity in these cells [27]. Also GABA is taken up and metabolized by the cultured astrocytes, although at considerably lower rates [13,24,25]. These findings, together with a high glutaminase activity in synaptosomes [8,9], are obviously in support of the concept of a transfer of glutamate and GABA from neurons to astrocytes and of glutamine in the opposite direction. A recent finding of a quite intense low affinity uptake of glutamine into the cultured astrocytes and of a rather high glutaminase activity in these cells [16,26] casts, however, some doubt on the hypothesis of an intense glutamine flow back from astrocytes to neurons. This is especially so since no high affinity uptake of glutamine seems to occur in synaptosomes [3,23,33] although such an uptake has been reported into brain prisms [2], a preparation in which only the neuronal uptake is supposed to persist [22].

Whether or not glutamine preferentially is taken up into neurons, or maybe into certain (e.g., glutamatergic) neurons is of crucial importance to confirm or refute the hypothesis of a quantitatively important flow of glutamine from astrocytes to neurons. Therefore, the kinetics for glutamine uptake were, in the present study, investigated in neuronal perikarya obtained by gradient centifugation, in cultured cerebral neurons, and in cultured cerebellar, presumably glutamatergic, granule cells, and the uptake into brain prisms was reinvestigated. The latter preparation was used as a source of relatively undamaged brain tissue, i.e., a sort of control, with the added advantage of a probably mainly neuronal uptake [22], and the reason to study uptake both into cultured neurons and into neuronal perikarya obtained by gradient centrifugation was to use neuronal preparation with widely different sources of error.

Primary cultures highly enriched in cerebellar granule cells were obtained as described by Messer [19]: Cerebella of 7-day-old DBA mice were removed, cut into small cubes, exposed to 0.05% trypsin in Puck's solution at 37°C for 10 min and centrifuged for another 10 min at 900 × *g* in this medium. The pelleted cells were seeded in 60 mm polylysine coated* Falcon plastic petri dishes (one dish per brain) in a modified Eagle's MEM (4 times enriched in vitamins: 2 times enriched in amino acids except glutamine, kept at 2 mM), which also contained increased concentrations of potassium (24.5 mM) and glucose (30 mM) as well as 1 μg *p*-aminobenzoic acid and 100 μU insulin per ml (cf. ref. 19). The medium was changed after 4—5 days, at which time the cultures were exposed for 24 h to 5×10^{-6} M cytosine-arabinoside (in medium) which led to a virtual disappearance of astrocytes [10]. Thereafter, the cultures were refed with medium without the mitotic inhibitor and used for uptake measurements at day 7. The cortical neurons were obtained from the brain hemispheres of 15-day-old DBA mouse embryos [10,29] in a similar manner, grown in an identical medium, exposed to cytosine-arabinoside for 24 h and used for uptake experiments at day 7. Neurons of several dif-

*By exposure to 50 μg/ml of polysine in 150 mM sodium borate overnight and subsequent thorough wash with sterile water and Hank's solution.

ferent morphological appearances were observed in these cultures indicating that no obvious selection of specific neurons had occurred. Bulk-prepared perikarya were obtained from adult Wistar rats as described by Hamberger and coworkers [11,12,33]. Brain prisms (0.1 × 0.1 × 0.5 mm) were prepared by aid of a McIlwain chopper from slices (0.5 mm thickness) cut from the lateral surface (one slice per hemisphere) of adult Swiss mice [28].

For measurements of the uptake of [^{14}C]glutamine (from New England Nuclear, Boston, MA), all preparations except brain prisms and neuronal perikarya were incubated in a modified MEM with 'normal' concentrations of potassium and glucose and no *p*-aminobenzoic acid or insulin. Brain prisms and perikarya were incubated in a saline phosphate-buffered (pH 7.4) medium containing 135 mM NaCl; 3.0 mM KCl; 1.0 mM $CaCl_2$; 0.6 mM $MgCl_2$; and 6 mM glucose. This difference will, if anything, lead to a relative overestimate of the uptake into brain prisms and perikarya since glutamine uptake is partly inhibited by certain amino acids [6]. The tissues were preincubated for 30 min in a medium with the selected glutamine concentration before the exposure to the labelled glutamine; uptake rates were calculated from the specific activity of the medium and expressed relative to protein content, measured by a conventional Lowry technique [17].

The time course for the uptake of ^{14}C-labelled glutamine into cultured

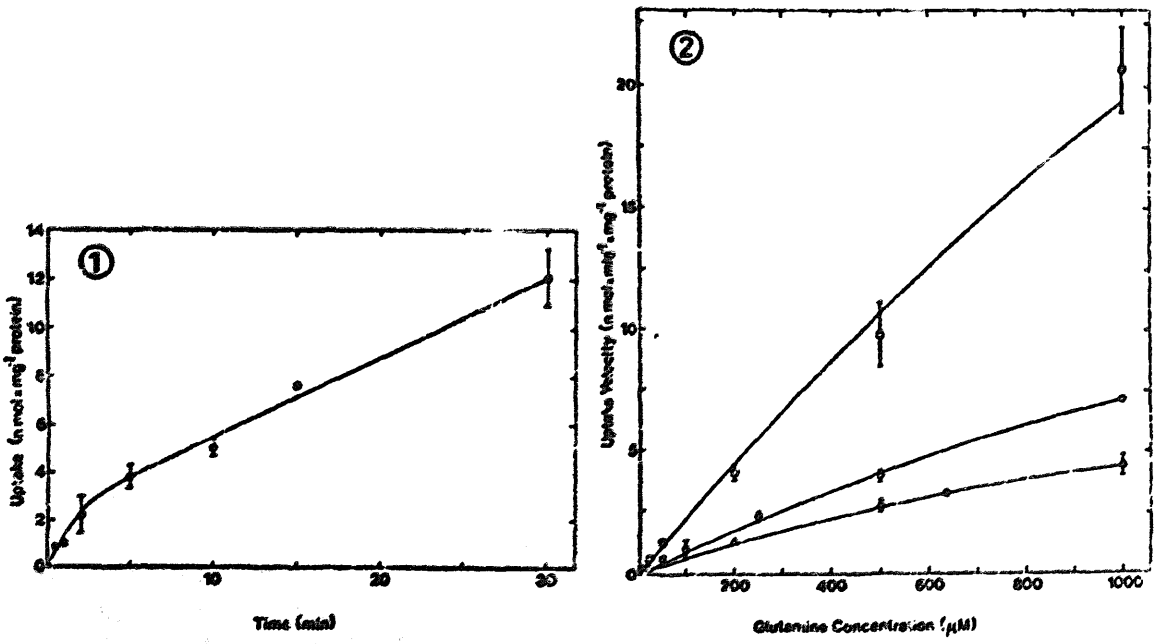

Fig. 1. Rate of glutamine uptake into cortical neurons as a function of time from the start of incubation, i.e., the exposure to ^{14}C-labelled glutamine (0.1 mM). Results with S.E.M. indicated by vertical bars are means of three experiments, whereas the 0.5-, 1- and 15-min values each shows a single experiment.

Fig. 2. Initial uptake rates for glutamine into brain prisms (□), clutured cortical neurons (○), and neuronal perikarya prepared by gradient centrifugation (△) as a function of the glutamine concentration in the incubation medium. Results are means of 4—5 experiments with S.E.M. indicated by vertical bars if extending beyond the symbols. The curves are drawn on the basis of computer analysis of the uptake rates as described in the text and in the legend of Table I.

TABLE I

K_m- AND V_{max}-VALUES WERE CALCULATED FROM INITIAL RATES OF GLUTAMINE UPTAKE AT GLUTAMINE CONCENTRATIONS BETWEEN 5 AND 1000 μM (5000 μM FOR BRAIN PRISMS AND 10,000 μM FOR ASTROCYTES) BY THE AID OF A UNIVAC 1110 COMPUTER. $V_{0.5\ mM}$ ARE THE MEASURED UPTAKE RATES AT THIS GLUTAMINE CONCENTRATION ± S.E.M. WITH NUMBER OF EXPERIMENTS IN PARENTHESES.

	K_m (μM)	V_{max} (nmol · min^{-1} · mg^{-1} protein)	$V_{0.5\ mM}$ (nmol · min^{-1} · mg^{-1} protein)
Brain prisms	5.0 ± 0.6[c]	117.2 ± 2.4[c]	9.8 ± 1.36 (5)
Neuronal perikarya	1.4 ± 0.2	10.8 ± 1.0	2.8 ± 0.21 (4)
Cortical neurons in primary cultures	3.0 ± 0.3	28.2 ± 1.9	3.9 ± 0.23 (4)
Cerebellar granule cells in primary cultures	0.7 ± 0.08	10.3 ± 0.8	4.4 ± 0.22 (2)
Synaptosomes[a]	0.26 ± 0.01	9	5.5
Astrocytes in primary cultures[b]	3.3 ± 0.5	50.2 ± 12.6	6.4 ± 0.63 (7)

[a] Indicates results published by Baldessarini and Yorke [3].
[b] Indicated results previously published by Schousboe et al. [26].
[c] S.E.M. for K_m and V_{max} were calculated on the basis of the means of the uptake rates at each concentration.

cortical neurons from a medium containing 0.1 mM glutamine is shown in Fig. 1. The uptake is almost rectilinear, i.e., occurs at the initial velocity, for 2 min; unless the glutamine is metabolized extremely rapidly it is also concentrative, since the content of labelled glutamine after less than one min exceeds 1 nmol/mg protein which, with a probable protein content of about 100 mg/g wet wt., corresponds to 0.1 μmol/g wet wt. or approximately to the medium concentration of 0.1 mM. Similar experiments (results not presented) showed concentrative uptakes into brain prisms, neuronal perikarya, and cerebellar granule cells in primary cultures; a concentrative net uptake into astrocytes has previously been described [26]. In all these preparations, initial uptake rates were observed for at least about 2 min and 2-min incubations were used for all subsequent experiments.

The glutamine uptakes into brain prisms, cortical neurons in primary cultures, and neuronal perikarya, as a function of the glutamine concentration is shown in Fig. 2, which suggests a saturable uptake following Michaelis-Menten kinetics, as described by the equation $V = V_{max}[1/(1 + K_m/S)]$. Computer analysis (using a Univac 1110 computer) was performed to determine the curves described by this equation which most closely fit to the experimental results. From these curves, the K_m- and V_{max}-values shown in Table I were determined. The highest V_{max} and the lowest affinity (highest K_m) were found in brain prisms. Both neuronal perikarya and cortical neurons showed considerably lower V_{max}-values; although also the K_m-values were somewhat decreased, the uptakes remained low affinity uptakes. Preliminary measurements and analysis of the glutamine uptake into the presum-

ably glutamatergic cerebellar granule cells showed a lower V_{max} than in the cortical neurons and a K_m of 0.7 mM (Table I): even this K_m is, however, almost one order of magnitude higher than the K_m-values of less than about 0.1 mM which are characteristic for high affinity uptakes. Uptake characteristics into synaptosomes have been determined by Baldessarini and Yorke [3] and remind us of those into cerebellar granule cells (Table I). K_m- and V_{max}-values for glutamine uptake into astrocytes in primary cultures have previously been determined by us [26], and are rather similar to those in the cortical neurons, but higher than those in the granule cells (Table I).

The extracellular glutamine concentration in the brain may be as high as 0.5 mM (cf. following paragraphs). The observed glutamine uptakes at this concentration are, therefore, indicated in the last column of Table I, which shows that the uptake into astrocytes is at least as high as into any neuronal preparation.

No neuronal high-affinity uptake of glutamine could be demonstrated in this study regardless whether neurons had been obtained by either culturing or gradient centrifugation, or whether neuronal uptake was studied in brain prisms. These findings are in agreement with previous observations that no high affinity uptake of glutamine occurs into synaptosomes [3,23,33] but at variance with the high affinity uptake of glutamine into brain prisms reported by Balcar and Johnson [2]. The results are probably representative for neurons in vivo. The only, remote, possibility that this might not be so is, if a deficient differentiation of the cultured cells *and* a mechanical damage of the neuronal perikarya and brain prisms both should abolish a possible high affinity uptake of glutamine without interfering with the low affinity uptake.

The observed low-affinity uptake of glutamine into neurons could be quite efficient on account of a probably rather high extracellular glutamine concentration, as reflected by a glutamine level of 0.5 mM in cerebrospinal fluid (see refs. 9,13,15,26 and 33). The similar uptake characteristics in astrocytes, which constitute about one-third of the cellular volume in the mammalian brain cortex [24], indicate, however, that a substantial amount of extracellular glutamine will be accumulated into astrocytes rather than into neurons. This uptake into astrocytes is known to represent a net uptake, not a homoexchange [26]* and obviously does *not* support the concept of a glutamine transport from astrocytes to neurons, compensating for the pronounced neuronal release of glutamate and GABA and the uptake of these two amino acids into astrocytes (see Introductory paragraph). The resulting conclusion must be that a net transfer of TCA constituents probably does occur from neurons to astrocytes. Such a net drain of TCA constituents from neurons would be feasible, provided it was compensated for by CO_2 fixation, a process known to occur quite actively in brain [32]. An intense net flow of glutamate from neurons to astrocytes might link these two cell types together in one metabolic compartment. Evidence for this concept and similarities between such a joint neuronal-astrocytic compartment and the 'small compartment' in metabolic compartmentation is discussed elsewhere [13].

*This is probably also true for the neuronal uptake which seems to be concentrative (Fig. 1).

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