Neuronal–glial metabolism under depolarizing conditions

A ^{13}C -n.m.r. study

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Time courses of incorporation of ^{13}C from ^{13}C -labelled glucose and/or acetate into the individual carbon atoms of amino acids, citrate and lactate in depolarized cerebral tissues were monitored by using ^{13}C -n.m.r. spectroscopy. There was no change in the maximum percentage of ^{13}C enrichments of the amino acids on depolarization, but the maxima were reached more rapidly, indicating that rates of metabolism in both glycolysis and the tricarboxylic acid cycle were accelerated. Although labelling of lactate and of citrate approached the theoretical maximum of 50 %, labelling of the amino acids was always below 20 %, suggesting that there is a metabolic pool or compartment that is inaccessible to exogenous substrates. Under resting conditions labelling of citrate and of glutamine from $[1-^{13}\text{C}]$ glucose was not detected, whereas both were labelled from $[2-^{13}\text{C}]$ acetate, which is considered to reflect glial metabolism. In contrast, considerable labelling of these two metabolites from $[1-^{13}\text{C}]$ glucose was observed in depolarized tissues, suggesting that the increased metabolism may be due to increased consumption of glucose by glial cells. The labelling patterns on depolarization from $[1-^{13}\text{C}]$ glucose alone and from both precursors ($[1-^{13}\text{C}]$ glucose plus $[2-^{13}\text{C}]$ acetate) were similar, which also indicates that the changes are due to increased consumption of glucose rather than acetate.

INTRODUCTION

We have previously used ^{13}C -labelled glucose and acetate to study glial–neuronal metabolic interactions in guinea-pig cerebral-cortical slices by ^{13}C -n.m.r. spectroscopy. Our findings are broadly in agreement with earlier ^{14}C radiotracer studies (see Van den Berg, 1973) in that under normal conditions glutamine is more highly labelled from acetate than from glucose (Badar-Goffer *et al.*, 1990). Our current view is that under normal conditions externally administered acetate is preferentially metabolized by glia whereas glucose, although ubiquitously metabolized, reflects primarily neuronal metabolism.

Glutamine appears to be a major precursor for the biosynthesis of the excitatory pool of neurotransmitter glutamate (Bradford *et al.*, 1978; Hamberger *et al.*, 1979; Ward *et al.*, 1983) and possibly also of the inhibitory neurotransmitter 4-aminobutyrate (Tapia & Gonzalez, 1978; McGeer *et al.*, 1983; Paulsen *et al.*, 1988). The site of synthesis of glutamine is attributed to the glial cells, where the enzyme glutamine synthase was found to be predominantly located (Martinez-Hernandez *et al.*, 1977).

In order for the neuronal network to function smoothly, these neurotransmitters, after being released into the synaptic cleft, are removed by uptake processes, many of which occur in glial cells. Glutamate was found to be taken up into retinal glia by an Na^+ -dependent glutamate carrier (Brew & Attwell, 1987), and in synaptosomes glutamate, aspartate and 4-aminobutyrate were all rapidly labelled from $[^{15}\text{N}]$ glutamine (Yudkoff *et al.*, 1989). Thus there is a constant flow of metabolites between glia and neurons that maintains normal brain function and replenishes neurotransmitter pools.

Using ^{13}C -n.m.r. spectroscopy to study compartmentation offers the advantages of resolving individually labelled carbon atoms, together with the different isotopomers (London, 1988). The relative proportions of the isotopomers can provide additional information on neuronal–glial interactions and flow of

label between metabolites as has been described by Cerdan *et al.* (1990).

In the study reported here, cerebral-cortical slices were depolarized in order to stimulate metabolism and presumably therefore also the flow of metabolites between neurons and glia. The labelling patterns, percentage enrichment and isotopomer composition of metabolites derived from $[^{13}\text{C}]$ glucose and $[^{13}\text{C}]$ acetate were studied and compared with those obtained under normal conditions.

One of the questions which remained unresolved in our previous studies (Badar-Goffer *et al.*, 1990) was the source of the unexpectedly highly labelled citrate observed in slices incubated with $[^{13}\text{C}]$ acetate but not with $[^{13}\text{C}]$ glucose under normal conditions. It was not known whether this was due to an increased pool size of citrate or to a genuinely higher enrichment. In the work presented here, this has been examined and a comparison of the labelling of citrate in normal and depolarized tissues has been performed.

MATERIALS AND METHODS

Chemicals

^{13}C -labelled precursors were obtained from Omicron Biochemical, Ithaca, NY, U.S.A. NADH, citrate lyase (EC 4.1.3.6), lactate dehydrogenase (EC 1.1.1.27) and malate dehydrogenase (EC 1.1.1.37) were from the Sigma Chemical Co., Poole, Dorset, U.K. All other chemicals were AnalaR grade from BDH Chemicals, Poole, Dorset, U.K.

Tissue preparation

Guinea-pig cerebral-cortical slices were prepared as described in Badar-Goffer *et al.* (1990). The slices were suspended in the incubation medium, which contained NaCl (124 mM), KCl (5 mM), KH_2PO_4 (1.2 mM), MgSO_4 (1.2 mM), CaCl_2 (1.2 mM), NaHCO_3 (26 mM) and glucose (10 mM), and gassed with O_2/CO_2

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(19:1) at 37 °C. They were washed several times in gassed medium at 37 °C and then incubated in fresh gassed medium for 20 min to restore their maximum metabolic state (McIlwain & Bachelard, 1985). The slices were then transferred to media containing 5 mM-[1-¹³C]glucose alone, 5 mM-[1-¹³C]glucose with 5 mM-[2-¹³C]acetate or 5 mM-[2-¹³C]acetate with unlabelled 5 mM-glucose. Depolarization was induced with 40 mM-KCl. Samples of the slices, incubated under control or depolarizing conditions, were withdrawn after the time intervals shown in the Results section, and neutralized deproteinized extracts were prepared as described previously (Badar-Goffer *et al.*, 1990).

Analyses

The n.m.r. conditions and calculation of ¹³C percentage enrichment were as described in Badar-Goffer *et al.* (1990). However, in that paper, a saturation factor of 1.0 for the internal dioxan standard was implicitly assumed. This did not affect the relative ¹³C enrichments of the different metabolites, but it did affect the absolute calculated values. For the results reported in this paper we measured the *T*₁ value for dioxan after addition to tissue extracts and found it to be 6.9 s, corresponding to a saturation factor of 2.06. The results for absolute percentage ¹³C enrichment described here are therefore lower than in Badar-Goffer *et al.* (1990) and we believe them to be correct. Citrate and lactate were measured using the enzymic spectrophotometric methods described by Lowry & Passonneau (1972). Amino acids were analysed in samples removed from the incubation media, at the same times as tissue samples were taken, using an LKB 4400 amino acid analyser.

RESULTS

The spectrum obtained from slices incubated with 5 mM-[2-¹³C]acetate and unlabelled 5 mM-D-glucose under depolarizing conditions (40 mM-K⁺) is shown in Fig. 1. The pattern of the resonances observed is similar to that observed under normal conditions (6.2 mM-K⁺). However, there is a difference in the calculated percentage ¹³C enrichment, in that under normal conditions the ¹³C enrichment of glutamine from acetate is always higher than that of glutamate (Badar-Goffer *et al.*, 1990), whereas here with high K⁺ the reverse situation is observed.

A comparison of spectra obtained from slices incubated with [1-¹³C]glucose under normal and depolarizing conditions is presented in Figs. 2(a) and 2(b) respectively. The main differences between the two spectra are the presence of the resonances of citrate C2/C4 and glutamine C4 in the spectra of the depolarized tissues that are not detected under normal conditions.

The spectra obtained from slices incubated with both precursors, 5 mM-[1-¹³C]glucose and 5 mM-[2-¹³C]acetate, under depolarizing conditions (Fig. 3) are essentially similar to those obtained from slices depolarized in the presence of [1-¹³C]glucose alone. The percentage ¹³C enrichments shown in Table 1 confirm this.

The percentage ¹³C enrichment of the n.m.r.-observable metabolites normally reaches a maximum at 20 min under depolarizing conditions, which is faster than that observed under control conditions (Table 1). For example, the enrichment of the C4 of glutamate from [1-¹³C]glucose reaches 15–20% at 20 min, which is similar to the maximal ¹³C enrichment of glutamate observed at 45 min under resting conditions. These results indicate that the metabolism of [¹³C]glucose is accelerated by depolarizing conditions but that the maximum ¹³C enrichments are unchanged, suggesting that the rates of metabolism in both glycolysis and the tricarboxylic acid cycle are increased under these conditions.

The spectra obtained under depolarizing conditions with [1-

¹³C]glucose as precursor exhibit very intense lactate resonances; this is to be expected as lactate formation is known to increase in such conditions. This increase with K⁺ is Na⁺-dependent, which is also observed when glycolysis is increased by electrical stimulation (McIlwain & Bachelard, 1985). It can be seen in the spectra (Figs. 2b and 3) that the high lactate production is accompanied by an alanine peak that is not detectable under resting conditions (Fig. 2a). Both lactate and alanine are derived from pyruvate. Although the resonances corresponding to lactate and alanine under depolarizing conditions are much higher than under resting conditions, there is no increase in their maximum ¹³C enrichments (Table 1). This would be expected as [1-¹³C]-glucose is the sole precursor of these metabolites and the resonances therefore increase only as a result of their larger pool sizes (Table 2). This is reinforced by the ¹H-n.m.r. spectra of one of the depolarized extracts (Fig. 4), where the lactate peak and its

Fig. 1. ¹³C-n.m.r. spectrum of a guinea-pig brain slice extract labelled from [2-¹³C]acetate and depolarized with 40 mM-K⁺.

Slices were incubated in the presence of 5 mM unlabelled glucose and 5 mM-[2-¹³C]acetate with 40 mM-K⁺ to induce depolarization. Slices were removed at 30 min and a neutralized HClO₄ extract was prepared as previously described (see Badar-Goffer *et al.*, 1990). EDTA (4 mM) was added to the samples. The ¹³C-n.m.r. spectrum was recorded at 100.62 MHz, with a nominal 90° pulse with broadband decoupling. Blocks of 4000 transients were acquired and an exponential line-broadening of 3 Hz was applied. The chemical shifts are relative to dioxan at 67.4 p.p.m. and were assigned to: 1, serine C3 (tentative); 2, EDTA; 3, glutamate C2; 4, glutamine C2; 5, aspartate C2; 6, citrate C2/C4; 7, 4-aminobutyrate C2; 8, succinate C2/C3 (tentative); 9, glutamate C4; 10, glutamine C4; 11, glutamate C3; 12, acetate C2.

Fig. 2. ^{13}C -n.m.r. spectra of guinea-pig brain slice extracts labelled from $[1-^{13}\text{C}]\text{glucose}$ under control (a) and depolarizing (b) conditions with 40 mM-K^+

Slices were incubated in the presence of $5\text{ mM-[}^{13}\text{C]glucose}$ for 30 min. Other experimental details are as for Fig. 1. Chemical shifts were assigned to: 1, serine C3 (tentative); 2, EDTA; 3, glutamate C2; 4, glutamine C2; 5, aspartate C2; 6, EDTA; 7, citrate C2/C4; 8, malate C3 (tentative); 9, 4-aminobutyrate C4; 10, aspartate C3; 11, 4-aminobutyrate C2; 12, glutamate C4; 13, glutamine C4; 14, glutamate C3; 15, glutamine C3; 16, 4-aminobutyrate C3; 17, lactate C3; 18, alanine C3.

Fig. 3. ^{13}C -n.m.r. spectrum of a guinea-pig brain slice extract labelled from $[2-^{13}\text{C}]\text{acetate}$ and $[1-^{13}\text{C}]\text{glucose}$ and depolarized with 40 mM-K^+

Slices were incubated in the presence of $5\text{ mM-[}^{13}\text{C]glucose}$ and $5\text{ mM-[}^{13}\text{C]acetate}$ for 30 min. Other experimental details are as for Fig. 1. Chemical shifts were assigned to: 1, serine C3 (tentative); 2, EDTA; 3, glutamate C2; 4, glutamine C2; 5, aspartate C2; 6, EDTA; 7, citrate C2/C4; 8, malate C3 (tentative); 9, 4-aminobutyrate C4; 10, aspartate C3; 11, 4-aminobutyrate C2; 12, glutamate C4; 13, glutamine C4; 14, glutamate C3; 15, glutamine C3; 16, 4-aminobutyrate C3; 17, *N*-acetylaspartate (tentative); 18, lactate C3; 19, alanine C3.

satellites are clearly discernible; calculation of the ratios of the satellite areas, due to $J_{\text{C-H}}$ coupling, to the total area of the lactate peaks, provides a direct measurement of ^{13}C enrichment,

which in this case was 45%. Although this method can theoretically be applied to all observable metabolites, in practice it is not straightforward for the ^1H resonances of, e.g., glutamate,

Table 1. ^{13}C enrichment of selected metabolites from $[1-^{13}\text{C}]\text{glucose}$ and $[2-^{13}\text{C}]\text{acetate}$ under control and depolarizing conditions

^{13}C enrichment (%) of the amino acids was calculated as described in Badar-Goffer *et al.* (1990). Samples were removed from a guinea-pig brain slice preparation incubated with 5 mM- $[1-^{13}\text{C}]\text{glucose}$ and/or 5 mM- $[2-^{13}\text{C}]\text{acetate}$ in the presence of either 6 mM- K^+ (control) or 40 mM- K^+ to induce depolarization. Abbreviations: U.D., undetectable; N.D., not determined. Control values are means $\pm$ S.D. ($n = 4$); depolarization values are from three experiments.

Time of incubation (min)	^{13}C enrichment (%)					
	Glutamate C4	Glutamate C2	Glutamine C4	4-Aminobutyrate C2	Alanine C3	Lactate C3
Precursor: $[1-^{13}\text{C}]\text{glucose}$						
Control						
15	3.6 $\pm$ 1.4	0.4 $\pm$ 0.3	U.D.	6.0 $\pm$ 2.1	U.D.	22.0 $\pm$ 3.2
30	6.0 $\pm$ 1.4	1.0 $\pm$ 0.2	U.D.	10.8 $\pm$ 3.2	U.D.	24.6
45	12.5 $\pm$ 3.4	3.2 $\pm$ 0.8	U.D.	17.0 $\pm$ 5.6	U.D.	N.D.
Depolarized						
10	8.6 $\pm$ 0.6	2.4 $\pm$ 0.3	2.7 $\pm$ 0.3	5.5 $\pm$ 2.0	13.7 $\pm$ 4.5	45.0
20	15.3 $\pm$ 0.3	6.0 $\pm$ 0.5	5.7 $\pm$ 0.3	12.1 $\pm$ 3.9	25.3 $\pm$ 3.5	43.1
30	15.2 $\pm$ 0.3	7.1 $\pm$ 0.7	5.0 $\pm$ 1.0	13.4 $\pm$ 3.6	21.3 $\pm$ 1.2	47.5
Precursor: $[1-^{13}\text{C}]\text{glucose}$ plus $[2-^{13}\text{C}]\text{acetate}$						
Control						
15	4.8 $\pm$ 1.6	0.7 $\pm$ 0.5	3.2 $\pm$ 0.2	2.9 $\pm$ 0.6	U.D.	N.D.
30	9.7 $\pm$ 0.8	2.1 $\pm$ 0.4	5.9 $\pm$ 0.1	6.2 $\pm$ 2.8	U.D.	N.D.
45	14.1 $\pm$ 1.6	4.3 $\pm$ 1.6	8.2 $\pm$ 0.8	13.7 $\pm$ 2.2	U.D.	N.D.
Depolarized						
10	5.0	0.8	1.8	4.5	5.5	N.D.
20	18.0	6.0	6.0	10.0	27.0	N.D.
30	18.3 $\pm$ 0.3	6.6 $\pm$ 0.9	7.1 $\pm$ 0.1	16.2 $\pm$ 3.2	17.5 $\pm$ 7.5	N.D.

Table 2. Metabolite pool sizes in depolarized tissues

Values of the amino acid pool sizes are given as means $\pm$ S.D., from five separate depolarization experiments. Amino acid concentrations were determined by amino acid analyses of tissue extracts and protein determinations were performed on the pellets retained after HClO_4 extraction. Abbreviation: U.D., undetectable. Data for non-depolarized control experiments are from Badar-Goffer *et al.* (1990).

Time of incubation (min)	Pool size ($\mu\text{mol}/100 \text{ mg}$ of protein)					
	Glutamate	Glutamine	4-Aminobutyrate	Aspartate	Alanine	Lactate
10	6.5 $\pm$ 2.8	3.2 $\pm$ 0.7	1.7 $\pm$ 0.4	2.1 $\pm$ 1.2	0.7 $\pm$ 0.4	1.9 $\pm$ 0.1
20	6.4 $\pm$ 0.8	2.6 $\pm$ 0.3	1.8 $\pm$ 0.5	1.1 $\pm$ 0.4	0.6 $\pm$ 0.1	3.1 $\pm$ 1.0
30	5.7 $\pm$ 0.2*	2.9 $\pm$ 0.9	1.7 $\pm$ 0.4	1.2 $\pm$ 0.3	0.8 $\pm$ 0.4	3.6 $\pm$ 1.2
Control						
15	10.1 $\pm$ 1.8	2.8 $\pm$ 1.1	1.5 $\pm$ 0.3	1.9 $\pm$ 0.3	U.D.	1.5 $\pm$ 0.1

* $P < 0.05$.

glutamine and 4-aminobutyrate, which, owing to restricted rotation, consist of many overlapping lines, making quantification difficult.

The spectra of Figs. 2(b) and 3 also show discernible resonances attributable to glutamine and citrate, which could be observed from the earliest time points. These are observed in both the single- ($[^{13}\text{C}]\text{glucose}$) labelled and double-labelled depolarization experiments and are of great interest because experiments performed under normal conditions showed that citrate is labelled from $[^{13}\text{C}]\text{acetate}$ but not from $[^{13}\text{C}]\text{glucose}$ (Badar-Goffer *et al.*, 1990). We have analysed enzymically the total pool of citrate in the guinea-pig brain slice extracts obtained from this study and those performed under normal conditions. The results of Table 3 show that there is no significant difference between the total citrate pool size in the different types of experiments. On the basis of these results we have been able to calculate the percentage ^{13}C enrichment of citrate in experiments where its resonance was visible. Under normal conditions the ^{13}C enrichment of citrate from $[2-^{13}\text{C}]\text{acetate}$ reaches a maximum of 15% whereas none

is detectable from $[1-^{13}\text{C}]\text{glucose}$. However, under depolarizing conditions enrichment from $[2-^{13}\text{C}]\text{acetate}$ was 10% and that from $[1-^{13}\text{C}]\text{glucose}$ was 32% (Table 3).

It may be noted that the substantial labelling of citrate was always accompanied by a high enrichment of glutamine C4. This occurred in acetate-labelling studies (normal conditions) and also in glucose-labelling studies (depolarizing conditions) and suggests that the observable citrate may be associated with glial metabolism. Labelling of citrate may therefore reflect a high rate of glial metabolism.

DISCUSSION

A primary objective of these double-labelling studies was to develop a method for simultaneous observation of neuronal and glial metabolism. The conclusion from the double-label studies performed under normal conditions with $[1-^{13}\text{C}]\text{glucose}$ and $[2-^{13}\text{C}]\text{acetate}$ was that the incorporation of label into glutamate represents mainly neuronal metabolism whereas incorporation

Fig. 4. ^1H n.m.r. spectrum of a guinea-pig brain slice extract labelled from $[2\text{-}^{13}\text{C}]\text{acetate}$ and $[1\text{-}^{13}\text{C}]\text{glucose}$ and depolarized with 40 mM-K^+

Slices were incubated in the presence of $5\text{ mM-[1-}^{13}\text{C}]\text{glucose}$ and $5\text{ mM-[2-}^{13}\text{C}]\text{acetate}$ for 20 min. Other experimental details are as for Fig. 1. The ^1H n.m.r. spectrum was recorded at 400 MHz, with a nominal 90° pulse with a water suppression pulse sequence. Blocks of 2000 transients were acquired and were Fourier-transformed with an exponential line-broadening of 1 Hz. The chemical shifts are relative to dioxan at 3.6 p.p.m. and were assigned to: 1, $\text{N}(\text{CH}_3)_3$ groups of choline, phosphocholine and α -glycerophosphocholine; 2, N-CH_3 groups of creatine and phosphocreatine; 3, 4-aminobutyrate $\gamma\text{-CH}_2$; 4, aspartate $\beta\text{-CH}_2$; 5, $\beta\text{-N-CH}_2$ acetylaspartate; 6, glutamate $\gamma\text{-CH}_2$; 7, 4-aminobutyrate $\gamma\text{-CH}_2$; 8, glutamate $\beta\text{-CH}_2$; 9, $\text{N-acetylaspartate CH}_3$; 10, acetate CH_3 (tentative). Lactate is the CH_3 of lactate. Inositol is the CHOH of inositol.

Table 3. Pool sizes and percentage ^{13}C enrichments of citrate

Citrate pool sizes are $\mu\text{mol}/100\text{ mg}$ of protein; ^{13}C enrichment (%) is the maximal percentage enrichment observed in spectra where the signal/noise ratio was sufficient for accurate measurement. The overall citrate concentration of $0.38 \pm 0.13\text{ }\mu\text{mol}/100\text{ mg}$ of protein ($n = 34$) compares well with the literature value of $0.33\text{ }\mu\text{mol}/100\text{ mg}$ of protein (Goldberg *et al.*, 1966). Abbreviation: U.D., undetectable.

Precursor	Control (6 mM-K^+)			Depolarization (40 mM-K^+)		
	Citrate pool size ($\mu\text{mol}/100\text{ mg}$)	n	Enrichment (%)	Citrate pool size ($\mu\text{mol}/100\text{ mg}$)	n	Enrichment (%)
$[1\text{-}^{13}\text{C}]\text{Glucose}$	0.42 ± 0.16	7	U.D.	0.44 ± 0.09	5	32
$[2\text{-}^{13}\text{C}]\text{Acetate}$	0.36 ± 0.11	6	15	0.32 ± 0.07	3	10
$[1\text{-}^{13}\text{C}]\text{Glucose plus } [2\text{-}^{13}\text{C}]\text{acetate}$	0.33 ± 0.17	9	14	0.42 ± 0.14	4	45

into glutamine represents glial metabolism (Badar-Goffer *et al.*, 1990). When double-label studies were performed under depolarizing conditions it at first appeared that the time course was merely an accelerated version of that observed under resting conditions. However, when a single-label $[1\text{-}^{13}\text{C}]\text{glucose}$ depolarization study was performed it was evident that glucose metabolism by glia was selectively stimulated, and that in the double-labelling experiments the contribution of acetate to glutamine and citrate had become small compared with that from glucose. Thus it may be concluded that, although double-label studies with precursors that label the same carbon atoms may be useful under resting conditions for the study of neuronal-glial interactions, this combination cannot be used under perturbed metabolic states without prior knowledge, from single-labelling studies, of the relative contribution (percentage enrichment) of each labelled precursor to the subsequently labelled resonances.

In studies with glucose labelled on one carbon atom, the

theoretical maximal labelling of lactate, acetyl-CoA and subsequent metabolites is 50%. Here, labelling of lactate and of citrate was close to this, but the maximum labelling calculated for the amino acids was below 20%. The peak ratios, of glutamate C4 to glutamate C2 + C3 (Fig. 3), did not change between 20 and 30 min, which also provides evidence that maximal labelling of glutamate has been achieved. Fitzpatrick *et al.* (1990) also reported maximum ^{13}C enrichment within 20 min *in vivo*. Our results on the low percentage enrichment of the amino acids suggest that there is a pool of these metabolites that is inaccessible to exogenous substrates. This may be related to numerous reports on release of labelled glutamate and 4-aminobutyrate from synaptosomes, which have led to the view that the cytoplasmic pool is rapidly labelled from exogenous glucose, or is in rapid exchange with exogenous amino acids, in contrast with the vesicular pool, which turns over more slowly (for review see Nicholls, 1989).

The efflux of amino acids into the incubation medium was

measured; the results obtained during the preincubation period demonstrated clearly that there is a steady efflux of glutamine into the medium, which we calculated to be up to 35 nmol/min per g of tissue. The results obtained after further incubation were not sufficiently consistent to assess if there is any difference in efflux to the media from normal and depolarized tissues. However, it has previously been found that elevated K^+ concentration does stimulate the efflux of glutamine into the medium (Cotman & Hamberger, 1978).

Glutamine is present in the blood and cerebrospinal fluid at high concentrations of around 0.5 mM (Plum, 1974); *in vivo*, it easily crosses the blood-brain barrier (Oldendorf, 1971) and *in vitro* it continuously moves out of slices into the superfusing medium. In a depolarization study performed by Hamberger *et al.* (1979) it was found that the synthesis and efflux of glutamate labelled from glucose was 3–6-fold larger when unlabelled glutamine was present in the medium than in the presence of glucose alone. The glutamate released to the medium was calculated to be 25% derived from glucose and 70% from glutamine. Furthermore, whereas the glutamate content of the tissue during depolarization with glucose alone slowly decreased, it remained constant when glutamine was also present in the medium. Our results (Table 2) also show a small significant decrease in the tissue content of glutamate on depolarization.

Several other studies cited in the Introduction have established the importance of glutamine as a substrate for the releasable glutamate pool. In our preparation each slice may be considered an integral system where neurons are tightly surrounded by, and in close contact with, glia; neuronal metabolism may thus be undisturbed by the absence of external glutamine if sufficient glutamine is available from synthesis *de novo* in the glia. However, in future studies it will be important to assess any effects of the presence of 0.5 mM external glutamine on these labelling patterns.

It has been previously suggested that one possible reason that certain substrates are selectively metabolized to glutamine in the central nervous system is that they are transported only into the compartment containing glutamine synthase. Shank & Campbell (1984) reported that citrate, acetate, proline, 2-oxoglutarate, leucine, 4-aminobutyrate and glutamate were all metabolized to glutamine, but they found evidence for high-affinity carriers in astrocyte membranes only for 4-aminobutyrate and glutamate. Therefore compartmentation cannot be due solely to a localization of transport carriers, and it is possible that intact central-nervous-system tissues are structurally arranged in a way that promotes selective uptake by astrocytes when these substrates are supplied exogenously (Shank & Campbell, 1984). What presumably occurs is that under normal conditions the glial

uptake and metabolism of glucose is not as efficient as that of endogenous acetate, which more closely resembles a breakdown (or recycling) product. The results presented here suggest that the contribution of glucose to glial metabolism increases on depolarization.

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REFERENCES

- Badar-Goffer, R. S., Bachelard, H. S. & Morris, P. G. (1990) *Biochem. J.* **266**, 133–139
- Bradford, H. F., Ward, H. K. & Thomas, A. J. (1978) *J. Neurochem.* **30**, 1453–1459
- Brew, H. & Attwell, D. (1987) *Nature (London)* **327**, 707–709
- Cerdan, S., Kunnecke, B. & Seelig, J. (1990) *J. Biol. Chem.* **265**, 12916–12926
- Cotman, C. W. & Hamberger, A. C. (1978) *Amino Acids as Transmitters*, pp. 379–412, Plenum Press, New York
- Fitzpatrick, S. M., Hetherington, H. P., Behar, K. L. & Shulman, R. G. (1990) *J. Cereb. Blood Flow Metab.* **10**, 170–179
- Goldberg, N. D., Passonneau, J. V. & Lowry, O. H. (1966) *J. Biol. Chem.* **241**, 3997
- Hamberger, A. C., Chiang, G. H., Nylen, E. S., Scheff, S. W. & Cotman, C. W. (1979) *Brain Res.* **168**, 513–530
- London, R. E. (1988) *Prog. Nucl. Magn. Reson. Spectrosc.* **20**, 337–383
- Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 157 and 200, Academic Press, New York
- Martinez-Hernandez, A., Bell, K. P. & Norenberg, M. D. (1977) *Science* **195**, 1356–1357
- McGeer, E. G., McGeer, P. L. & Thompson, S. (1983) in *Glutamate, Glutamine and GABA in the Central Nervous System* (Hertz, L., Kvamme, E., McGeer, E. G. & Schousboe, A., eds.), pp. 3–17, Alan Liss, New York
- McIlwain, H. & Bachelard, H. S. (1985) *Biochemistry and the Central Nervous System*, 5th edn., p. 69, Churchill-Livingstone, Edinburgh
- Nicholls, D. G. (1989) *J. Neurochem.* **52**, 331–341
- Oldendorf, W. H. (1971) *Am. J. Physiol.* **221**, 1629–1635
- Paulsen, R. G., Odden, E. & Fonnum, F. (1988) *J. Neurochem.* **51**, 1294–1299
- Plum, C. M. J. (1974) *J. Neurochem.* **23**, 595–600
- Shank, R. P. & Campbell, G. LeM. (1984) *J. Neurosci.* **4**, 58–69
- Tapia, R. & Gonzalez, R. M. (1978) *Neurosci. Lett.* **10**, 165–169
- Van den Berg, C. J. (1973) in *Metabolic Compartmentation in the Brain* (Balazs, R. & Cremer, J. E., eds.), pp. 137–166, Macmillan, London
- Ward, H. K., Thanki, C. M. & Bradford, H. F. (1983) *J. Neurochem.* **40**, 855–860
- Yudkoff, M., Zaleska, M. M., Nissim, I., Nelson, D. & Erecinska, M. (1989) *J. Neurochem.* **53**, 632–640

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