The oxidation of glutamine and glutamate in relation to anion transport in enterocyte mitochondria

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1. The oxidation of L-glutamate and L-glutamine by enterocyte mitochondria was supported by malate. The stimulation of the rate of oxidation of the two amino acids by small amounts of added malate was 93% and 76% respectively. This could not be accounted for by the oxidation of the small amounts of malate added. 2. Amino-oxyacetate added initially inhibited malate-supported oxidation of L-glutamate by 81% and that of L-glutamine by 38%. The inhibition of L-glutamate oxidation was partially reversed by L-glutamine. 3. The dicarboxylate-carrier inhibitor 2-phenylsuccinate inhibited the malate-supported oxidation of both amino acids, but appeared to be slightly stimulatory to L-glutamine oxidation when added initially. The inhibition of L-glutamate oxidation was reversed by L-glutamine. 4. The mitochondrial uncoupler FCCP (carbonyl cyanide *p*-trifluoromethoxyphenylhydrazine) inhibited malate-supported oxidation of L-glutamate by 78% when added initially. The oxidation of L-glutamine was completely inhibited. However, the uncoupler stimulated the oxidation of both amino acids when added finally. 5. Pyruvate inhibited aspartate synthesis when either of these amino acids was the main substrate, alanine being synthesized. There was no effect on O₂ uptake. 6. Mitochondria did not swell in KCl solution, but swelled rapidly in water. Mitochondrial swelling in potassium phosphate and potassium acetate solutions was activated by valinomycin and to a lesser extent by the further addition of FCCP. With potassium malate, swelling was mainly activated by phosphate. 7. The swelling of enterocyte mitochondria in potassium glutamate was slow. In glutamine solution, mitochondrial swelling was greater and appeared to be enhanced by the initial presence of small amounts of phosphate.

The metabolic pathways available to amino acids in most specialized mammalian organs are now well founded (reviewed by Evered, 1981). Compared with liver, brain, kidney and muscle, interest in the metabolism of the small intestine has only been kindled during the last decade. Windmueller & Spaeth (1974) discovered that L-glutamine was a major respiratory fuel in the small intestine of the rat. L-Glutamine accounted for about one-third of the CO₂ produced by vascularly perfused intestine, which led to several further investigations at essentially two levels of metabolic study. These included perfusion studies (Windmueller & Spaeth, 1975, 1978, 1980; Hanson & Parsons, 1977) and studies at the cellular level using isolated enterocytes from the rat or chick

small intestine (Watford *et al.*, 1979; Porteous, 1980; Ampon, 1980). Pinkus & Windmueller (1977) further found that the major L-glutamine-utilizing enzyme was phosphate-dependent glutaminase, localized in the enterocyte mitochondrion.

The work of Müller & Leuthardt (1950), Braundstein (1957), Krebs & Bellamy (1960), Borst (1962), Kovačević (1971) and others showed that there were two likely pathways by which the amino acids L-glutamate and L-glutamine were oxidized by the mitochondrion. When L-glutamate was the substrate, the transamination pathway, which yields L-aspartate and 2-oxoglutarate, was apparently preferred. However, when L-glutamine was the substrate the glutamate dehydrogenase pathway was preferred (Kovačević, 1971). Kovačević (1971) suggested that when exogenously added L-glutamate was the substrate, the transamination pathway was preferred because of a favourable

Abbreviation used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazine.

localization of L-aspartate:2-oxoglutarate aminotransferase in the mitochondrion. He further proposed that L-glutaminase, the first enzyme to act on L-glutamine, was located deeper inside the mitochondrion, such that L-glutamate produced from L-glutamine *in situ* was oxidized via the glutamate dehydrogenase pathway.

In the present study the use of a powerful inhibitor of aminotransferases, amino-oxycacetate, permitted investigation of the relative importance of these two pathways when either of these two amino acids was the substrate for enterocyte mitochondria. The characteristics of anion transport in enterocyte mitochondria were also investigated by means of the swelling technique (Chappell & Haarhoff, 1967).

Experimental

Animals

In all experiments fed male rats of the Wistar albino strain, weighing 190–220 g, were used.

Chemicals

L-Glutamine, L-glutamate, valinomycin, rotenone, amino-oxycacetate, FCCP, ADP (disodium salt), pyruvic acid, L-malic acid, L-aspartic acid, L-alanine, glycine and bovine serum albumin (fraction V) were from Sigma Chemical Co., Poole, Dorset, U.K. 2-Phenylsuccinate was from Aldrich Chemical Co., Gillingham, Dorset, U.K. The chemicals were stored desiccated at 0–4°C. All other chemicals used were of the highest purity obtainable. Solutions were prepared in double-distilled water. FCCP, valinomycin and rotenone were dissolved in ethanol and stored at –20°C. Mitochondrial substrates and inhibitors were neutralized to pH 7.0 and stored at –20°C when not in use. L-Glutamine solutions were prepared freshly.

Mitochondrial experiments

Mitochondria were prepared as described by Masola & Evered (1984). Unless otherwise stated, the mitochondrial preparation medium consisted of 250 mM-sucrose, 5 mM-Tris and 2 mM-EGTA, adjusted to pH 7.4; the incubation medium consisted of 25 mM-sucrose, 75 mM-mannitol, 100 mM-KCl, 10 mM-Tris, 10 mM-K₂HPO₄, 1 mM-EGTA and 0.1% albumin, adjusted to pH 7.5. The adjustments of pH were made with dilute HCl.

The functional integrity of the mitochondria was assessed by measuring the respiratory control ratio (Chance & Williams, 1956). Mitochondria were utilized when this ratio was above 4 with a 5 mM-L-glutamate/1 mM-malate couple as the substrate. O₂ consumption was monitored by using an O₂ electrode (Chappell, 1964). The final volume for

each incubation was 3.3 ml, containing about 2 mg of mitochondrial protein. Mitochondrial swelling was monitored at 520 nm with a Pye-Unicam SP.1800 double-beam spectrophotometer, with temperature maintained at 30°C. Conditions were as stated in the text.

Assays

The mitochondria (approx. 2.5 mg of protein) were incubated in open conical flasks placed in a shaking water bath maintained at 37°C. The reactions were terminated after 10 min by adding conc. aq. sulphosalicylic acid solution to a final concentration of 3% (w/v). The deproteinized samples were centrifuged at 700 *g*_{max} in an MSE Centaur bench centrifuge for 3 min to remove protein, which was dissolved in 0.5 M-NaOH for protein assays. Supernatants and protein solutions were stored at –20°C until required for analysis.

Amino acids in the supernatant solutions were assayed by automatic analysis by using a Locarte amino acid analyser and the method recommended by the makers. Sodium buffers were used for elution. Protein was determined either by the method of Lowry *et al.* (1951) or with the biuret method (Gornall *et al.*, 1949), with bovine serum albumin as the standard.

Results

Effects of malate

The effects of malate on oxidation of glutamate and glutamine by enterocyte mitochondria were investigated by presenting malate at such concentrations that it minimally stimulated respiration; a suitable concentration was 0.1 mM. In Fig. 1 and Table 1(a), malate support of oxidation of glutamate and glutamine was demonstrated; it stimulated their oxidation by 93% and 76% respectively. The magnitude of the stimulation of O₂ uptake by 0.1 mM-malate in the presence of glutamate and glutamine was about 3 times that with 0.1 mM-malate alone as the substrate. Thus the increase in O₂ uptake on addition of malate could not be due mainly to direct support for respiration by malate. With glutamine as the main substrate, and in the presence of 0.1 mM-malate, there was a further 17% stimulation of O₂ by 0.25 mM-glutamate. However, a similar concentration of glutamate had no effect on glutamine oxidation in the absence of malate (result not shown). Malate was readily oxidized by enterocyte mitochondria, and endogenous respiration in absence of added ADP could be observed.

Kovačević (1971) reported that glutamine was not utilized by rat liver mitochondria except at unphysiological concentrations. We found (D. F. Evered & B. Masola, unpublished work) that this was not due to a malate requirement. We have

Fig. 1. Uptake of O_2 by enterocyte mitochondria in the presence of glutamate (Glu) and glutamine (Gln), and the effects of malate (Mal)

Conditions are described in the Experimental section. Abbreviation: Mito, mitochondria.

further noted that glutamine was not utilized by rat heart mitochondria up to concentrations of 20 mM, but the malate effect has not been tested (B. Masola, unpublished work).

Effects of amino-oxyacetate

The powerful aminotransferase inhibitor amino-oxyacetate inhibited malate-supported oxidation of both glutamate and glutamine (Figs. 2a and 2b, and Table 1b). Inhibition was most severe when the inhibitor was added initially (Table 1b), being 81% and 38% with glutamate and glutamine respectively as the main substrates. However, when added finally inhibition was lower, indicating a build-up of tricarboxylic acid-cycle intermediates. The results with glutamine were in contrast with those obtained by Kovačević (1971) with mitochondria from ascites tumour, rat liver and kidney, where amino-oxyacetate had no effect on glutamine oxidation, but were similar to the findings of Dennis *et al.* (1976) working with rat

brain mitochondria. However, glutamine partially reversed the inhibition of malate-supported glutamate oxidation by amino-oxyacetate, which was in agreement with the findings of Kovačević (1971).

Effects of 2-phenylsuccinate

2-Phenylsuccinate, an inhibitor of the dicarboxylate carrier (Chappell *et al.*, 1968; Palmieri *et al.*, 1971), was used to investigate the route of malate stimulation of oxidation of glutamate and glutamine. When added finally (Figs. 2c and 2d, and Table 1b), the inhibition of malate-supported oxidation of glutamate and glutamine was 28% and 24% respectively. However, when added initially, inhibition was increased to 34% for glutamate, but there was a 3% stimulation with glutamine. The findings with glutamine as the main substrate were contradictory. Glutamine (1 mM) reversed the inhibition of glutamate oxidation by 2-phenylsuccinate.

Fig. 2. Uptake of O_2 by enterocyte mitochondria in the presence of glutamate (Glu) and glutamine (Gln), and the effects of amino-oxyacetate (AOA), 2-phenylsuccinate (Ph.succ) and FCCP. Conditions are described in the Experimental section. Abbreviation: Mal, malate.

Table 1. *O₂* uptake by enterocyte mitochondria in the presence of glutamate and glutamine and the effects of malate, amino-oxyacetate, 2-phenylsuccinate and FCCP

Sequence of additions was glutamate (Glu) or glutamine (Gln), mitochondria, ADP and malate (Mal), with inhibitors added initially or finally. The ADP concentration was 0.8mM. Rates of State-3 respiration (nmol of O₂ consumed/min per mg of protein at 30°C) were means ± S.D. for the numbers of individual experiments shown in parentheses. Conditions were as described in the Experimental section.

(a)		Substrates	Rates of State-3 respiration			
		0.1 mM-Mal	5.1 ± 0.4 (6)			
		5 mM-Glu	17.4 ± 2.5 (6)			
		5 mM-Glu + 0.1 mM-Mal	33.6 ± 3.4 (8)			
		5 mM-Gln	17.4 ± 1.5 (6)			
		5 mM-Gln + 0.1 mM-Mal	30.6 ± 4.1 (8)			
		5 mM-Gln + 0.1 mM-Mal + 0.25 mM-Glu	35.7 ± 2.1 (4)			
(b)			Rates of State-3 respiration			
		Substrates . . .	5 mM-Glu/0.1 mM-Mal		5 mM-Gln/0.1 mM-Mal	
	Inhibitors					
	5 mM-Amino-oxyacetate		Added initially	Added finally	Added initially	Added finally
	10 mM-2-Phenylsuccinate		6.3 ± 0.7 (4)	14.5 ± 1.4 (4)	18.9 ± 1.7 (4)	20.8 ± 1.3 (4)
	1.2 μM-FCCP		22.3 ± 1.6 (4)	24.3 ± 1.2 (4)	23.3 ± 1.8 (4)	31.4 ± 2.5 (4)
			7.5 ± 0.5 (4)	36.9 ± 2.7 (4)	0 (4)	38.4 ± 3.2 (4)

Effects of FCCP

A suitable concentration of the mitochondrial uncoupler FCCP was established by titration (Fig. 2e). Maximum uncoupler activity was found between 1.2 and 3 µM. Above 4 µM the uncoupler was inhibitory. FCCP stimulated malate-supported oxidation of both amino acids when added finally (Figs. 2f and 2g, and Table 1b). However, when added initially (Table 1b), the uncoupler completely stopped glutamine oxidation, whereas that of glutamate was inhibited by 78%. This suggested that, when tricarboxylic acid-cycle intermediates were allowed to build up intramitochondrially, electrogenic steps involved in oxidation of glutamate and glutamine were masked.

Mitochondrial swelling

The mitochondrial swelling technique has been used to study ion penetration of the mitochondrial membrane (Chappell & Crofts, 1966; Chappell & Haarhoff, 1967). The swelling experiments are illustrated in Fig. 3. Additions of valinomycin, FCCP, malate and phosphate were of small volume, such that dilution effects were largely eliminated. Mitochondria, as expected, did not swell in KCl, but swelled rapidly in water. In potassium acetate and potassium phosphate solutions (Fig. 3a) enterocyte mitochondria swelled when the ionophore valinomycin was added. FCCP caused a further stimulation of swelling. In potassium malate solution the mitochondria did not swell appreciably until phosphate was added. FCCP did not seem to have any effect in this case.

The swelling of enterocyte mitochondria in

Fig. 3. Swelling of enterocyte mitochondria in water, in 150mM solutions of KCl, potassium acetate, potassium phosphate, potassium malate, potassium glutamate, and in 150mM-glutamine (+ 75 mM-KCl)

Where indicated by arrows, the final concentrations of the additions were as follows: 2mM-phosphate, 3mM-malate, 1.2 µM-FCCP and 1.5 µg of valinomycin (Val)/ml. In each case 10mM-Tris/HCl (pH 7.4), 0.8mM-EGTA, 10 µg of rotenone and 2.1mg of mitochondrial protein were present. The final volume was 3.3ml. Mixing in the cuvette and its return to the spectrophotometer were as rapid as possible.

Table 2. *Studies on enterocyte mitochondrial metabolites*

Mitochondrial preparation medium consisted of 250mM-sucrose, 10mM triethanolamine, 1mM-EGTA, pH 7.4. Incubation medium contained 120mM-KCl, 10mM-K₂HPO₄, 10mM-triethanolamine, 0.1% albumin, pH 7.5. The ADP concentration was 2.5mM. Mitochondria were incubated at 37°C as described in the Experimental section. Amino acid concentrations in the supernatants were determined by analysis with a Locarte automatic analyser. Abbreviation: Pyr, pyruvate; for others see Table 1. Results are means $\pm$ S.D. for the numbers of individual experiments shown in parentheses.

Substrates		Products (nmol produced/min per mg of protein)	
		Aspartate	Alanine
1. 1mM-Mal	(9)	—	—
2. 5mM-Glu + 1mM-Mal	(9)	7.21 $\pm$ 0.5	1.67 $\pm$ 0.28
3. 5mM-Glu + 5mM-Pyr + 1mM-Mal	(9)	3.91 $\pm$ 0.38	7.69 $\pm$ 0.43
4. 0.5mM-Glu + 1mM-Mal	(6)	1.91 $\pm$ 0.25	*
5. 5mM-Gln + 1mM-Mal	(6)	3.88 $\pm$ 0.31	*
6. 5mM-Gln + 1mM-Mal + 0.5mM-Glu	(9)	6.7 $\pm$ 0.29	0.7 $\pm$ 0.07
7. 5mM-Gln + 5mM-Pyr + 1mM-Mal + 0.5mM-Glu	(9)	2.4 $\pm$ 0.21	2.89 $\pm$ 0.23

* Alanine was present, but the peaks were too small to be integrated accurately.

potassium glutamate (Fig. 3b) was slow, being negligibly stimulated by valinomycin. Subsequent additions of malate and FCCP had no effect. In glutamine supplemented with KCl, mitochondrial swelling was greater and valinomycin produced a significant stimulation of swelling. However, the subsequent addition of FCCP and malate had no effect. Swelling of mitochondria in glutamine (+ KCl) appeared to be enhanced by the initial presence of small amounts of phosphate (Fig. 3b). Subsequent addition of malate inhibited swelling, whereas the further addition of FCCP had no effect.

Aspartate synthesis

Small amounts of glycine were synthesized in these experiments. The major amino acid synthesized during the oxidation of glutamate and glutamine was aspartate, whose synthesis was inhibited by pyruvate (Table 2). Aspartate production was affected by the degree of coupling. Alanine was also synthesized. The difference in aspartate synthesis between the glutamine/malate/trace-glutamate combination and the glutamine/malate combination was 2.82 nmol/min per mg of protein at 37°C. This, compared with the 1.91 nmol of aspartate synthesized/min per mg via the trace glutamate/malate couple, seems to indicate that glutamate was in part enhancing aspartate formation, possibly by exchange on the glutamate (H⁺)/aspartate antiporter.

Discussion

Pathways of oxidation of glutamate and glutamine in enterocyte mitochondria

Glutamate can be oxidized by animal mitochondria by two pathways, the glutamate dehydrogenase pathway and the transamination pathway.

With rat heart and pigeon breast-muscle mitochondria the transamination pathway seemed obligatory, whereas in the liver both pathways existed (Borst, 1962). When glutamate was the substrate, mitochondria, including those from ascites-tumour cells and kidney (Kovačević, 1971), used the transamination pathway preferentially. When liver, kidney or ascites-tumour mitochondria respired with glutamine as the substrate, amino-oxyacetate had no effect on oxygen uptake (Kovačević, 1971), indicating a preference for the glutamate dehydrogenase pathway.

Kovačević (1971) proposed that, with added glutamate as substrate, the transamination pathway was preferred because of a favourable localization of L-aspartate:2-oxoglutarate aminotransferase in the mitochondrion. With glutamine as the substrate, it was proposed that the glutamate dehydrogenase pathway was preferred because the glutaminase was deeper inside the mitochondrion, such that glutamate produced was relatively inaccessible to the transamination pathway. This proposition was supported by the finding that, when glutamate oxidation was inhibited by amino-oxyacetate, the addition of glutamine stimulated O₂ uptake. Our results demonstrated that a similar situation exists in enterocyte mitochondria in that amino-oxyacetate inhibited glutamate oxidation by 81% and that this inhibition was partially reversed by the addition of glutamine. In contrast with the findings of Kovačević (1971), we found that amino-oxyacetate inhibited glutamine oxidation by 38% in enterocyte mitochondria. Compared with the 81% inhibition with glutamate as the substrate, it appears that, although the glutamate dehydrogenase pathway is prominent, the transamination pathway plays a major role in the oxidation of glutamine in enterocyte mito-

chondria. In this respect it is worth noting the significant production of aspartate during malate-supported glutamine oxidation (Table 2).

Anion transport in enterocyte mitochondria

Anion transport in mitochondria has been extensively reviewed by Chappell (1968), Meijer & Van Dam (1974), Fonyó *et al.* (1976), La Noue & Schoolwerth (1979) and more recently by Kovačević & McGivan (1983).

Enterocyte mitochondria swelled in potassium acetate and potassium phosphate in the presence of valinomycin and in the absence of FCCP. FCCP did, however, cause a further stimulation of swelling. Phosphate can be transported into mitochondria electroneutrally in exchange for OH^- ions or by proton co-transport. Since mitochondria do not swell in medium containing valinomycin, K^+ and phosphate in the absence of a mechanism for removing protons from the interior (Mitchell & Moyle, 1969), the swelling of enterocyte mitochondria in the absence of FCCP indicated a leakage of protons on addition of valinomycin. This could have been due to osmotic shock. Valinomycin had, however, little effect on enterocyte mitochondria suspended in potassium malate solution until catalytic phosphate was added. This was taken as evidence for the existence in enterocyte mitochondria of the dicarboxylate carrier, which catalyses electroneutral malate translocation in exchange for intramitochondrial phosphate (Chappell & Haarhoff, 1967).

At physiological pH values, the mitochondrial membrane is not easily permeable to glutamate because it carries a negative charge. Transport of glutamine, which is electrically neutral, should be faster. The slow swelling in the presence or absence of valinomycin indicated that glutamate was penetrating the enterocyte mitochondrion. This was in agreement with the slow O_2 uptake in the absence of added malate. The electroneutral glutamate/ OH^- antiporter has been characterized by various workers (Azzi *et al.*, 1967; Meijer *et al.*, 1972). This antiporter is known to be active in rat brain mitochondria (Dennis *et al.*, 1976), in liver mitochondria, where it is involved in urea synthesis (Meijer *et al.*, 1975), and in heart mitochondria. Its activity in kidney mitochondria appears to be low (Kovačević *et al.*, 1970; Schoolwerth *et al.*, 1983). Our results suggested that this carrier may also be active in enterocyte mitochondria. This proposition was supported by the inability of FCCP to inhibit completely glutamate oxidation.

The second carrier likely to be involved in glutamate transport was the glutamate (H^+)/aspartate antiporter (Azzi *et al.*, 1967), known to be electrogenic (La Noue & Tischler, 1974; La Noue *et al.*, 1974). This carrier is linked to the 2-

oxoglutarate antiporter in the Borst (1963) shuttle, which is primarily for transferring reducing equivalents from the cytosol to the mitochondrion. When used for glutamate oxidation or for the generation of aspartate for urea synthesis, as in the liver, the glutamate (H^+)/aspartate antiporter is probably linked to the dicarboxylate carrier and hence to the phosphate carrier, with malate providing oxaloacetate for aspartate synthesis. The presence of this carrier in enterocyte mitochondria can be inferred from the severe inhibition of malate-supported oxidation of glutamate by FCCP and the dependence of aspartate production on the degree of coupling in our preparations. The inhibition of malate-supported oxidation of glutamate by 2-phenylsuccinate and amino-oxyacetate was indirect evidence of the existence of a variant of the Borst (1963) or malate-aspartate shuttle described earlier. Attempts to demonstrate this cascade by the swelling technique (Fig. 3b) were unsuccessful. This was not surprising with rotenone-inhibited mitochondria, where there was no energy source. Even if an energy source such as tetramethylphenylenediamine/ascorbate or succinate was provided, the efflux of aspartate would nullify the osmotic effects of glutamate entry. Furthermore the presence of valinomycin would destabilize membrane potentials.

In glutamine supplemented with KCl and in the presence of valinomycin, the swelling of enterocyte mitochondria was pronounced. Even in the absence of valinomycin, mitochondria swelling was appreciable. The initial presence of phosphate appeared to enhance swelling, but the stimulatory effect of valinomycin was largely nullified. If the activity of mitochondrial phosphate-dependent glutaminase regulates glutamine influx, then the phosphate effect can be easily explained. Although a glutamine/glutamate antiporter has been proposed for renal mitochondria (Chappell *et al.*, 1972), this is now regarded as untenable. Kovačević (1977) suggested that glutamine entry into kidney mitochondria was by a uniporter. Since glutamate is a potent inhibitor of mitochondrial phosphate-dependent glutaminase, two mechanisms have been proposed for its efflux from kidney mitochondria. One is electroneutral, where glutamate exits with a proton (La Noue & Schoolwerth, 1979), or in exchange for hydroxyl ions (Kovačević & McGivan, 1983), and the other is electrogenic (Kovačević, 1975). Kovačević & Bajin (1982) have provided evidence of the regulatory role of glutamate and 2-oxoglutarate on glutamine transport in liver mitochondria.

In enterocyte mitochondria, where glutamine is an obligatory respiratory fuel, the situation may be different. Glutamate generated by glutamine hydrolysis would be removed by oxidation. The

swelling of mitochondria in glutamine (+KCl) showed that glutamine entry was by a uniporter. This was supported by the slow oxidation of glutamine in the absence of malate. The activating effect of valinomycin on swelling in the absence of FCCP can be explained by assuming that the membrane had become leaky to protons, allowing for an exchange with K^+ ions in the absence of the proton ionophore. The hydrolysis of glutamine in the mitochondrion would lead to a lowering of pH and an inhibition of phosphate-dependent glutaminase, whose pH optimum was between 7.6 and 9.2 (Pinkus & Windmueller, 1977). Ammonia, by formation of NH_4^+ , could not compensate for this, as that ion would not leave the mitochondrion easily. Protons would therefore have to leave by coupled respiration, or, in our swelling experiments, by exchange for K^+ .

It was not clear whether or not malate support for glutamine oxidation was through malate entering the mitochondria via the dicarboxylate carrier and hence stimulating the transamination pathway. The results with 2-phenylsuccinate were equivocal and appeared to suggest glutamine protection of the dicarboxylate carrier. Small amounts

of glutamate stimulated malate-supported oxidation of glutamine. Aspartate production was also stimulated by small amounts of glutamate during malate-supported oxidation of glutamine. This stimulation could not be accounted for by aspartate generated from the transamination of oxaloacetate by the small amounts of glutamate added (Table 2). These effects of glutamate could be explained by assuming that external glutamate was exchanging for aspartate on the glutamate(H^+)/aspartate antiporter. This would relieve the inhibition of glutamine oxidation via the transamination pathway that would result from intramitochondrial accumulation of aspartate. The oxidation of glutamine in enterocyte mitochondria may therefore be heavily dependent on tight coupling to remove both protons generated from glutamine hydrolysis and those resulting from the operation of the glutamate(H^+)/aspartate antiporter. This could explain the sensitivity to FCCP. A possible scheme for events occurring during malate and glutamate-supported oxidation of glutamine is illustrated in Fig. 4. We cannot, however, explain the inhibition by malate of swelling in glutamine (+KCl) in the presence of phosphate. Further

Fig. 4. Possible events occurring during the oxidation of glutamine by the enterocyte mitochondrion

Key: 1, dicarboxylate carrier; 2, glutamate/aspartate antiporter; 3, malate dehydrogenase; 4, L-aspartate:2-oxoglutarate aminotransferase; PDG, phosphate-dependent glutaminase; GDH, L-glutamate dehydrogenase; 2-OG, 2-oxoglutarate; ~, electrogenic pump; ----, transamination route of glutamine oxidation.

work is clearly needed on the mechanisms of transport and oxidation of glutamine and glutamate in enterocyte mitochondria. Of special interest is malate support of glutamine oxidation.

Pyruvate inhibition of aspartate synthesis during the oxidation of glutamate and glutamine

Pyruvate inhibited aspartate synthesis during the oxidation of glutamate and glutamine by enterocyte mitochondria (Table 2). Similar results were obtained by Kovačević (1971), using kidney, liver and ascites-tumour-cell mitochondria, and by Dennis & Clark (1978), using brain mitochondria respiring on glutamate as the substrate. This phenomenon is thought to be due to the competition for oxaloacetate by pyruvate through acetyl-CoA (Dennis & Clark, 1978). Pyruvate, however, had no effect on O₂ uptake, which means that the oxidation of acetyl-CoA compensated for the inhibition of amino acid oxidation via the transamination pathway. We confirmed that pyruvate has no inhibitory effect on O₂ uptake when heart mitochondria respire with glutamate as the substrate (B. Masola, unpublished work).

Site of alanine synthesis in the intestinal mucosa

Alanine synthesis in the intestinal mucosa mainly utilizes pyruvate derived from lactate and glucose (Windmueller & Spaeth, 1978). Little alanine was synthesized by enterocyte mitochondria respiring with glutamine in the absence of added pyruvate, which is consistent with the finding that pyruvate is mainly derived from other sources than glutamine itself.

Hanson & Parsons (1980) have suggested that the main site of alanine synthesis in the intestinal mucosa is the cytosol, as little alanine aminotransferase activity is associated with the particulate fraction (Volman-Mitchell & Parsons, 1974). The metabolite experiments reported here (Table 2) have shown that isolated enterocyte mitochondria have a significant capacity for alanine synthesis, especially from exogenous glutamate when pyruvate is present.

The rates of alanine and aspartate synthesis might well be higher, as results were obtained with a medium system now regarded as unsuitable (Masola & Evered, 1984). If the proposition by Hanson & Parsons (1980) is correct, that glutamate derived from glutamine passes to the cytosol, where it transaminates pyruvate to form alanine, then there would have to be a mechanism for the removal of glutamate from the mitochondria. In this respect, the glutamate(H⁺)/aspartate antiporter would be unavailable because of its irreversibility owing to its electrogenicity. Alternatively, glutamate could exit electroneutrally or electrogenically, as described above.

Although we do not claim that all alanine is synthesized in the mitochondria, a simple scheme would be where pyruvate enters the mitochondrion and is transaminated by glutamate derived from glutamine. This area clearly needs further investigation.

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