

The Regulation of Glutamate Metabolism by Tricarboxylic Acid-Cycle Activity in Rat Brain Mitochondria

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(Received 22 August 1977)

1. The interrelationship of metabolism of pyruvate or 3-hydroxybutyrate and glutamate transamination in rat brain mitochondria was studied. 2. If brain mitochondria are incubated in the presence of equimolar concentrations of pyruvate and glutamate and the K^+ concentration is increased from 1 to 20 mM, the rate of pyruvate utilization is increased 3-fold, but the rate of production of aspartate and 2-oxoglutarate is decreased by half. 3. Brain mitochondria incubated in the presence of a fixed concentration of glutamate (0.87 or 8.7 mM) but different concentrations of pyruvate (0 to 1 mM) produce aspartate at rates that decrease as the pyruvate concentration is increased. At 1 mM-pyruvate, the rate of aspartate production is decreased to 40% of that when zero pyruvate was present. 4. Brain mitochondria incubated in the presence of glutamate and malate alone produce 2-oxoglutarate at rates stoichiometric with the rate of aspartate production. Both the 2-oxoglutarate and aspartate accumulate extramitochondrially. 5. Externally added 2-oxoglutarate has little inhibitory effect (K_i approx. 31 mM) on the production of aspartate from glutamate by rat brain mitochondria. 6. It is concluded that the inhibitory effect of increased C_2 flux into the tricarboxylic acid cycle on glutamate transamination is caused by competition for oxaloacetate between the transaminase and citrate synthase. 7. Evidence is provided from a reconstituted malate–aspartate (or Borst) cycle with brain mitochondria that increased C_2 flux into the tricarboxylic acid cycle from pyruvate may inhibit the re-oxidation of exogenous NADH. These results are discussed in the light of the relationship between glycolysis and reoxidation of cytosolic NADH by the Borst cycle and the requirement of the brain for a continuous supply of energy.

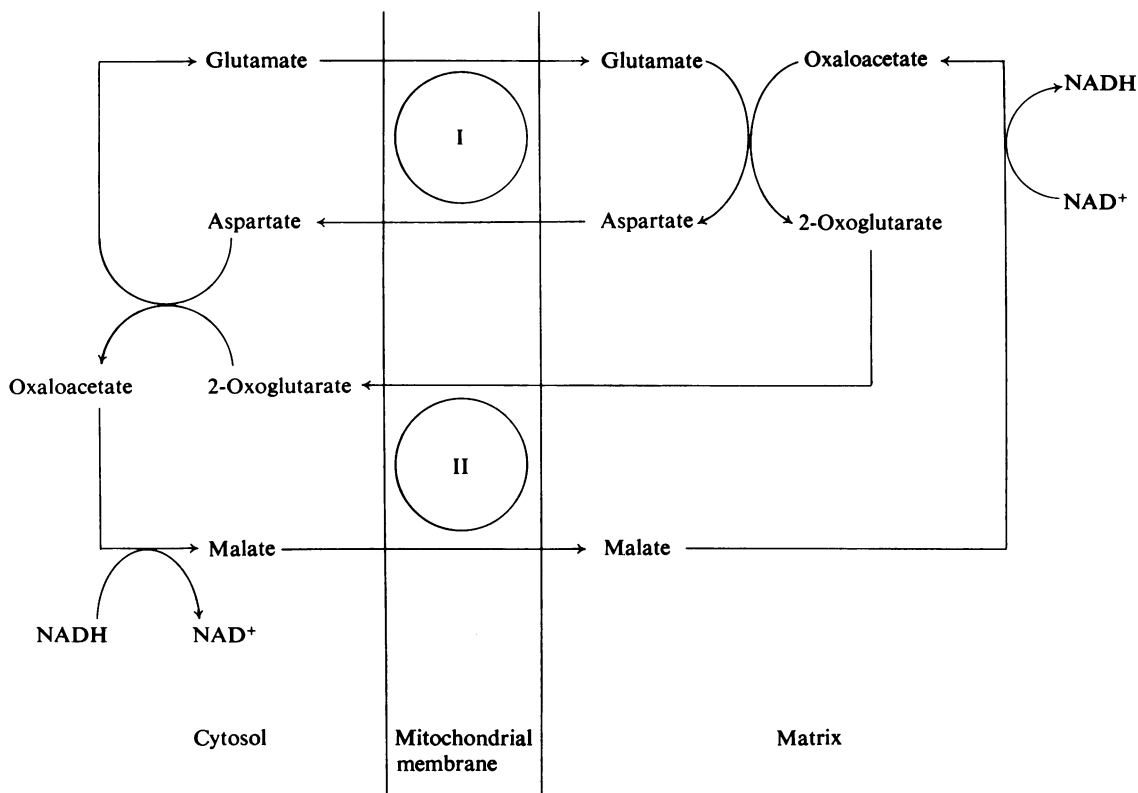
It is now well established that in preparations from both brain and heart glutamate metabolism is inhibited by oxidation of glucose or pyruvate (Haslam & Krebs, 1963; Balazs, 1965; Nicklas *et al.*, 1971; Benjamin & Quastel, 1975). This is in contrast with gluconeogenic tissues such as the liver and kidney, where a product of glutamate metabolism, 2-oxoglutarate, effectively inhibits pyruvate oxidation (Haslam, 1966).

Brain and liver, however, possess high specific activities of both glutamate–oxaloacetate transaminase and glutamate dehydrogenase (Dennis *et al.*, 1976), whereas heart mitochondria exhibit glutamate–oxaloacetate transaminase activity but only a very low glutamate dehydrogenase activity (Davis, 1968).

The interaction of glutamate metabolism with the tricarboxylic acid cycle is of importance because glutamate transamination is an integral part of the malate–aspartate shuttle, a primary mechanism for the indirect transfer of reducing equivalents from the cytosol to the mitochondrion (see Scheme 1). This shuttle, originally proposed by Borst (1963), and extensively investigated in both mitochondria and whole-

cell preparations from heart and liver (Robinson & Halperin, 1970; Lumeng & Davis, 1970; Rognstad & Katz, 1970; La Noue & Williamson, 1971; Berry & Kun, 1972; Cederbaum *et al.*, 1973; Williamson *et al.*, 1973; La Noue *et al.*, 1973; La Noue & Tischler, 1974; La Noue *et al.*, 1974*a,b*), requires the entry of malate and glutamate and the efflux of 2-oxoglutarate and aspartate from the mitochondria and is coupled to both the mitochondrial and cytosolic malate dehydrogenase and glutamate–oxaloacetate transaminase. Both the glutamate–oxaloacetate transaminase (Magee & Phillips, 1971) and the malate dehydrogenase (Lai, 1975) are distributed in brain in both the cytosolic and mitochondrial compartments, and attempts partially to reconstitute the Borst cycle with brain mitochondria have been made (Brand & Chappell, 1974*a*).

The present paper describes experiments designed to elucidate further the mechanisms whereby glutamate metabolism is controlled by tricarboxylic acid-cycle activity in brain mitochondria. The results are interpreted as suggesting that the interaction of glutamate metabolism and tricarboxylic acid-cycle



Scheme 1. *The malate-aspartate shuttle (or Borst cycle)*
I, Glutamate-aspartate translocase; II, 2-oxoglutarate translocase.

activity is associated with the control of the rate of mitochondrial reoxidation of the reduced nicotinamide nucleotides.

Methods

Animals

In all cases male adult rats (150–180 g), fed *ad lib.*, of the Wistar strain were used.

Chemicals

Glutamate-oxaloacetate transaminase (EC 2.6.1.1), hexokinase (EC 2.7.1.1), glutamate dehydrogenase (EC 1.4.1.3) and malate dehydrogenase (EC 1.1.1.37) were obtained from Boehringer Corp. (London), Lewes, Sussex, U.K. Hexokinase, before use, was dialysed for 6 h against 3×50 vol. of 50 mM-potassium phosphate, pH 7.0, and then stored frozen at -20°C . Pyruvic acid, supplied by Koch-Light Laboratories, Colnbrook, Bucks., U.K., was twice distilled under

vacuum and stored at -20°C before use. Ficoll was purchased from Pharmacia Fine Chemicals A.B., Uppsala, Sweden, and was dialysed before use for at least 5 h against 10 vol. of glass-distilled water. All other chemicals used were of the highest purity commercially available. Solutions were prepared in double-glass-distilled water and mitochondrial substrates neutralized with Tris base to pH 6.5–6.8.

Mitochondrial experiments

Rat brain mitochondria of non-synaptic origin were prepared by the method of Clark & Nicklas (1970). Evidence has been presented (Lai *et al.*, 1977) that these mitochondria are a population distinct from the mitochondria derived from synaptosomes, which were prepared by the method of Lai & Clark (1976).

Incubations were performed in a standard incubation medium, containing 100 mM-KCl, 75 mM-mannitol, 25 mM-sucrose, 5 mM-phosphate/Tris, pH 7.4, 20 mM-Tris/HCl, pH 7.4, 50 μM -EDTA adjusted to pH 7.4. In the cases where the K^+ concentration was

lowered to 1 mM the mannitol and sucrose concentrations were adjusted to maintain the same osmolality.

O₂-consumption rates were measured polarographically at 25°C by using a Clark-type oxygen electrode as described by Clark & Land (1974). In the experiments where metabolites were subsequently assayed, incubations were performed at 25°C with O₂ blown over the surface to provide aeration. State-3 conditions (Chance & Williams, 1956) were obtained by inclusion of a hexokinase trap (20 mM-glucose, 5 mM-MgCl₂, 1 mM-ADP and 1 unit of hexokinase/mg of mitochondrial protein, where a unit of activity, both here and elsewhere, is defined as that amount of enzyme required to convert 1 μmol of substrate/min). Samples of the incubation medium were removed at the timed intervals and added to HClO₄ (1.5 M) to produce a final concentration of 0.2–0.4 M-HClO₄. Neutralization was effected by titration of the acidified samples with 3 M-K₂CO₃ in 0.5 M-triethanolamine, and the precipitated protein and KClO₄ were removed by centrifugation at 15000g for 3 min.

Metabolite assays

Metabolites were determined either fluorimetrically or spectrophotometrically. The enzymic methods described by Williamson & Corkey (1969) were used for the assays of oxaloacetate, 2-oxoglutarate and aspartate and the method of Lowry *et al.* (1964) was used for pyruvate.

Protein was measured by the method of Gornall *et al.* (1949) or Lowry *et al.* (1951), with bovine plasma albumin as a standard.

Results

Previous work (Clark & Nicklas, 1970; Nicklas *et al.* 1971; Lai & Clark, 1976) has indicated that

pyruvate oxidation by rat brain mitochondria of both synaptic and non-synaptic origin is stimulated by K⁺. This effect of K⁺ is probably due to the sensitivity of the pyruvate dehydrogenase kinase to K⁺ and in particular the necessity for K⁺ in the inhibition of the kinase by ADP, this inhibition manifesting itself as an activation of the pyruvate dehydrogenase complex (Nicklas *et al.*, 1971; Roche & Reed, 1974). In addition it was observed that although K⁺ stimulated pyruvate oxidation most markedly, that of glutamate was also increased. Table 1 shows the results of experiments in which rat brain mitochondria of non-synaptic origin were incubated in the presence of both pyruvate and glutamate as substrates together with malate. The rates of substrate and oxygen utilization were measured in a medium containing 1 mM-K⁺, and then the K⁺ concentration was increased to 21 mM and the new rates of substrate and O₂ utilization were measured. In these experiments aspartate production was used as a measure of glutamate utilization (Balazs, 1965; Dennis *et al.*, 1976, 1977). Table 1 shows that on raising the K⁺ concentration from 1 to 21 mM, although the State-3 respiration rate decreased only marginally by 12%, the glutamate utilization decreased by more than 60%. If 3-hydroxybutyrate was used as an alternative substrate to pyruvate, a similar decrease in aspartate production was observed on increasing the K⁺ concentrations to that found for pyruvate. Again, however, the O₂ uptake decreased only by approx. 16%. In these experiments, owing to the high background concentration of pyruvate or 3-hydroxybutyrate, it was difficult to estimate reliably the rate of utilization of these two substrates, particularly at the low K⁺ concentration. Provisional estimates, however, suggest a 3-fold increase in the rate of pyruvate or 3-hydroxybutyrate utilization on addition of K⁺. This suggests therefore that the inhibition of glutamate transamination observed in these experi-

Table 1. Effect of K⁺ concentration on glutamate utilization and O₂ consumption in the presence of pyruvate or 3-hydroxybutyrate and malate

The incubations (final volume 10 ml) contained 4–7 mg of non-synaptic mitochondria oxidizing the indicated substrates in State 3 (Chance & Williams, 1956). Samples (0.75 ml) were removed for metabolite determination at 0, 1 or 2 min. At 3 min the K⁺ concentration of the medium was elevated (where indicated) from 1 to 21 mM by the addition of 1 M-KCl. Further samples were removed at 4, 6, 8 and 10 min. Aspartate-production rates were linear with time and changes in the rate resulting from an increased K⁺ concentration were exactly coincident with the addition of KCl. O₂-consumption measurements were performed polarographically in parallel 1 ml incubations. Values reported represent the means ± S.D. for duplicate measurements from at least three distinct experiments.

Experimental conditions		Metabolic flux rates	
Substrates	K ⁺ (mM)	Aspartate production (nmol/min per mg of mitochondrial protein)	O ₂ uptake (ng-atoms/min per mg of mitochondrial protein)
1 mM-Glutamate + 1 mM-pyruvate + 5 mM-malate	1	27 ± 6	79 ± 8
	21	10 ± 2	70 ± 10
1 mM-Glutamate + 1 mM-D-3- hydroxybutyrate ± 5 mM-malate	1	24 ± 5	72 ± 8
	21	10 ± 3	60 ± 6
1 mM-Glutamate + 5 mM-malate	100	27 ± 4	107 ± 8

ments by increased K^+ concentration is either a direct inhibition by K^+ of transamination or is associated with the increased production and further metabolism of C_2 units from either pyruvate or 3-hydroxybutyrate. It is clear, however, both from previous work (Nicklas *et al.*, 1971) and also from Table 1, that the former proposition is not tenable, since increasing K^+ to 100mM does not inhibit the utilization by brain mitochondria of glutamate and malate alone. Similar qualitative observations were seen with brain mitochondria of synaptic origin metabolizing a mixture of glutamate, pyruvate and malate under similar conditions to those in Table 1.

Further confirmation of the inhibition of glutamate transamination by increased acetyl-CoA utilization in the tricarboxylic acid-cycle is shown in Fig. 1 in which rat brain mitochondria (non-synaptic) were incubated in a medium containing 100mM- K^+ with increasing concentrations of pyruvate in the presence of two fixed concentrations of glutamate (■, 0.87mM; ●, 8.7mM). The choice of pyruvate concentrations was made on the basis of the kinetics of pyruvate-supported respiration in rat brain mito-

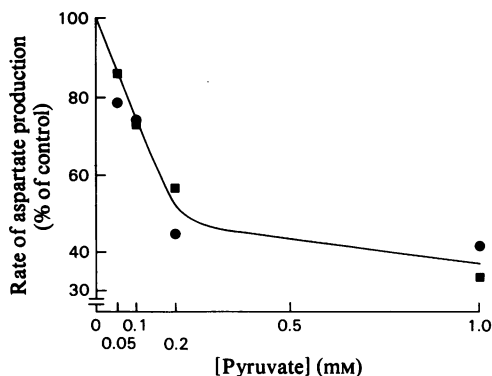


Fig. 1. Rat brain mitochondria oxidizing glutamate and malate: effect of increasing pyruvate metabolism on aspartate production

Rat brain mitochondria (non-synaptic; approx. 1.05mg of protein/ml) were incubated in the 100mM- K^+ medium (see the Methods section) under state-3 conditions in the presence of 2.5mM-malate + (■) 0.87mM- or (●) 8.7mM-glutamate and pyruvate at the indicated concentrations. Samples (0.2ml) for aspartate assay were removed at 1, 5, 10 and 15min from the assay, from which the rate of aspartate production was estimated. These rates were linear with time in all incubations. The rates of aspartate production were then plotted as percentages of the rate of aspartate production at specified glutamate concentrations in the absence of pyruvate. These rates of aspartate production were: at 0.87mM-glutamate, 44nmol/min per mg of protein, at 8.7mM-glutamate, 62nmol/min per mg of protein.

chondria (apparent K_m 40–80 μ M; Nicklas *et al.*, 1971; Dennis *et al.*, 1977), such that this particular concentration range would be predicted to result in a variable acetyl-CoA production, and in turn that the acetyl-CoA concentration so produced would be below saturation with respect to the citrate synthase (apparent K_m for acetyl-CoA approx. 17 μ M; Land & Clark, 1973). At each pyruvate concentration the rate of aspartate production was deduced from a time-course (four samples), and these rates are expressed as a percentage of the rate of aspartate production occurring in the absence of any pyruvate at that particular glutamate concentration. It is clear that as the pyruvate concentration is increased and hence the production of acetyl-CoA, the rate of transamination of glutamate decreases. This holds for both concentrations of glutamate (0.87 and 8.7mM), and although the absolute rates of transamination are different, when they are expressed as a percentage of the transamination rate in the absence of pyruvate, the inhibitions at both glutamate concentrations are quantitatively very similar, culminating in a maximal inhibition of the rate of transamination of over 60% at pyruvate concentrations of 1mM (cf. Balazs, 1965). Confirmation that the range of pyruvate concentrations used in the experiments of Fig. 1 does, as predicted, cause a variation in carbon flux through the tricarboxylic acid-cycle is shown in Fig. 2 where the accumulation of 2-oxoglutarate with time as a function of initial pyruvate concentration was measured. This shows an increasing rate of 2-oxoglutarate production with increased pyruvate concentration. The mean rate (\pm s.d.) of 2-oxoglutarate production at 1mM-pyruvate under State-3 conditions (Chance & Williams, 1956) by non-synaptic mitochondria was 6.6 ± 1.4 nmol/min per mg of protein ($n = 3$) and under State-4 conditions 2.4 ± 1.0 nmol/min per mg ($n = 3$).

The 2-oxoglutarate produced accumulated almost entirely in the extramitochondrial space, as experiments designed to permit assay of intramitochondrial 2-oxoglutarate gave values below the limits of detection. Synaptic mitochondria gave qualitatively similar results, the mean (\pm s.d.) State-3 rate of 2-oxoglutarate production being 2.3 ± 1.1 nmol/min per mg of protein ($n = 3$). It should be recalled here, however, that synaptic mitochondria have a pyruvate dehydrogenase activity approximately two-thirds that of non-synaptic mitochondria (Lai & Clark, 1976).

As previously indicated (Dennis *et al.*, 1977; Dennis & Clark, 1977) transamination appears to be the main route of glutamate metabolism in brain mitochondria. Hence the accumulation of 2-oxoglutarate in the experiments during which pyruvate was varied (Fig. 2) could conceivably have caused an inhibition of the glutamate-oxaloacetate transaminase, thus giving the decreased production shown in Fig. 1.

To assess the effects that 2-oxoglutarate might have

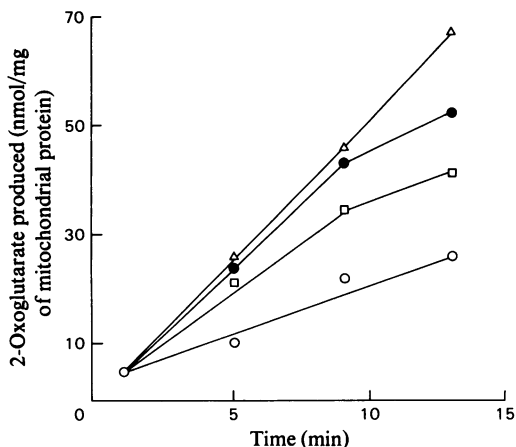


Fig. 2. 2-Oxoglutarate production by brain mitochondria at various pyruvate concentrations

Rat brain mitochondria of non-synaptic origin were incubated at a final concentration of approx. 1.5 mg of protein/ml in the 100 mM-K⁺ medium under State-3 conditions (see the Method section) in the presence of 2.5 mM-malate and various pyruvate concentrations: 0.125 mM (○); 0.25 mM (□); 0.5 mM (●); 1 mM (△). Samples were removed at timed intervals and assayed for 2-oxoglutarate. Control experiments showed that all the 2-oxoglutarate was extramitochondrial, the intramitochondrial concentrations being below the limits of detection. Each point represents the mean for at least duplicate determinations.

on glutamate transamination two types of experiments were carried out. First, brain mitochondria were incubated under State-3 conditions in the presence of glutamate and malate alone, and the accumulation of aspartate and 2-oxoglutarate was assessed (see Fig. 3). Secondly, the effect of added (exogenous) 2-oxoglutarate on glutamate transamination was studied (Fig. 4). In the first case (Fig. 3), with non-synaptic brain mitochondria, in the presence of 10 mM-glutamate and 2.5 mM-malate, a linear rate (with respect to time) of aspartate production of approx. 49 nmol/min per mg of protein was observed, together with an almost stoichiometric linear rate of 2-oxoglutarate accumulation (41 nmol/min per mg of protein). Similar observations were also made with brain mitochondria of synaptic origin, in which aspartate and 2-oxoglutarate production was 36 and 33 nmol/min per mg of protein respectively. Furthermore, if experiments are carried out in which mitochondria were separated from the medium and the intra- and extra-mitochondrial metabolite concentrations assessed (see Dennis *et al.*, 1976), then in all cases the accumulated 2-oxoglutarate was extramitochondrial, and intramitochondrial 2-oxoglutarate was undetectable (S. C. Dennis & J. B. Clark, un-

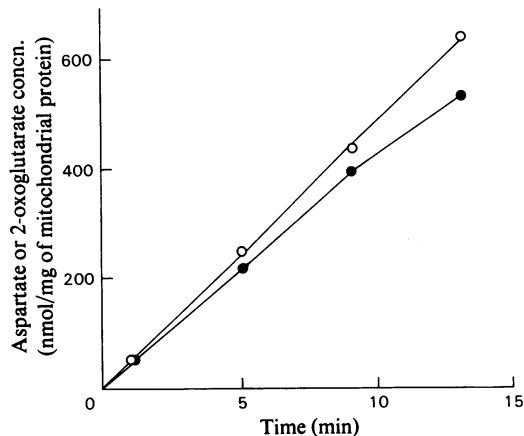


Fig. 3. Accumulation of 2-oxoglutarate by brain mitochondria metabolizing glutamate and malate

Rat brain mitochondria (non-synaptic; 1.7 mg of protein/ml) were incubated in 100 mM-K⁺ medium (see the Methods section) under State-3 conditions (Chance & Williams, 1956) in the presence of 10 mM-glutamate + 2.5 mM-malate. Samples were removed at the indicated times for aspartate (○) and 2-oxoglutarate (●) determinations. Points represent the mean values for at least three determinations.

published work). If, however, malate was absent from the experiments, then the rate of aspartate production was decreased 4-fold and 2-oxoglutarate accumulation was undetectable. In the case where exogenous 2-oxoglutarate (final concn. 1 mM) was added to non-synaptic brain mitochondria metabolizing various concentrations of glutamate and malate (Fig. 4), it is apparent that 2-oxoglutarate may act as an inhibitor of aspartate production. Further analysis of these results indicates, however, that the apparent K_i of 2-oxoglutarate with respect to glutamate is of the order of 31 mM. Both this experiment (Fig. 4) and those of Fig. 3, in which aspartate production was linear in spite of 2-oxoglutarate accumulating extramitochondrially, suggest that the accumulation of 2-oxoglutarate is not likely to be either of sufficient concentration or in the correct location (intramitochondrial) to give rise to the inhibition of aspartate production observed in the experiments of Fig. 1 (see also Table 1).

Further evidence to support this hypothesis is given in Fig. 5. In this case non-synaptic brain mitochondria were incubated in a medium containing 1 mM-K⁺ in the presence of pyruvate, glutamate and malate under State-3 conditions (Chance & Williams, 1956). Under these conditions pyruvate was utilized at 5.6 nmol/min per mg of protein, and aspartate and 2-oxoglutarate were produced at approx. 25 and 30 nmol/min per mg of protein respectively, during the first 5 min. After 6 min the K⁺ concentration was increased to 20 mM

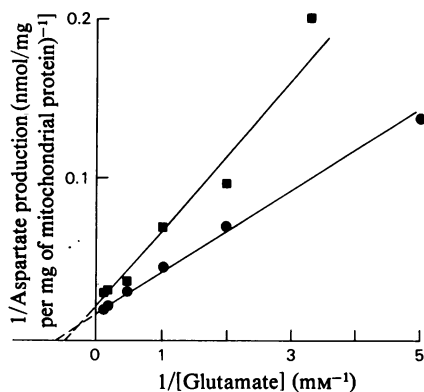


Fig. 4. Effect of exogenous 2-oxoglutarate on aspartate production from glutamate + malate by brain mitochondria

Non-synaptic rat brain mitochondria (approx. 0.7 mg of protein/ml) were incubated under State-3 conditions (Chance & Williams, 1956) in 100 mM-K⁺ medium (see the Methods section) in the presence of 2.5 mM-malate, various concentrations of glutamate, and where indicated (■) 1 mM-2-oxoglutarate was added (●, no further addition). The incubations were initiated by the addition of mitochondrial protein, and samples withdrawn for aspartate measurement at 0, 2, 4 and 6 min. Aspartate production was linear with time during this period and the reciprocals of these rates have been plotted against the reciprocals of the glutamate concentration. Computer analysis of the best-fit lines to these points give an apparent K_m for glutamate of 1.7 mM and an apparent K_i for 2-oxoglutarate for this system of 31 mM.

with a minimal change in volume, and almost immediately there was a decrease in the rate of aspartate and 2-oxoglutarate production, to 11 and 14 nmol/min per mg of protein respectively, coupled with an increase in pyruvate utilization to 15 nmol/min per mg of protein. This represents approximately a 3-fold increase in pyruvate utilization concomitant with a decrease in 2-oxoglutarate and aspartate production of greater than 50%. Hence there is a decrease in 2-oxoglutarate accumulation coincident with a decrease in glutamate transamination, again suggesting that 2-oxoglutarate is not involved in the decreased transamination occurring when K⁺ is added. The other component of transamination, oxaloacetate, was also measured during these experiments. However, at all stages of the incubation the concentrations were below the limits of detection (<0.05 nmol/mg of protein), despite the presence of 2.5 mM-malate.

Discussion

The inhibition of glutamate metabolism (transamination) by pyruvate metabolism in brain mitochondria may be easily demonstrated by stimulating

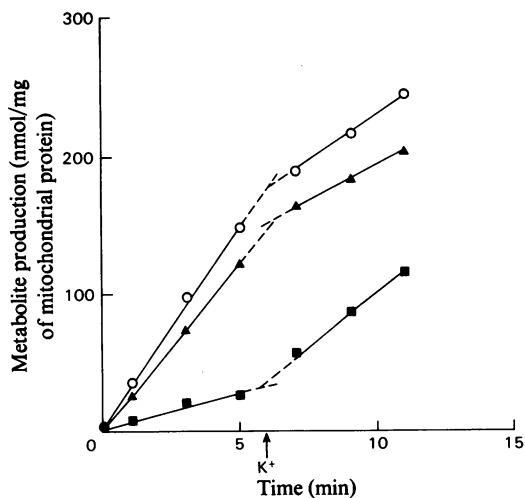


Fig. 5. Effect of K⁺ increase on pyruvate + glutamate + malate metabolism by rat brain mitochondria

Rat brain mitochondria (non-synaptic; 2.6 mg of protein/ml) were incubated in a medium containing 1 mM-K⁺ (see the Methods section) under State-3 conditions (Chance & Williams, 1956), in the presence of 1 mM-pyruvate, 1 mM-glutamate and 2.5 mM-malate. After 6 min incubation, the K⁺ concentration of the medium was increased to 20 mM. Samples were removed at timed intervals both before and after the K⁺ injection for measurement of pyruvate uptake (■) and aspartate (▲) and 2-oxoglutarate (○) production. These samples, after acid extraction (see the Methods section), were adjusted to pH 5 ± 0.2 for assay because of the instability of 2-oxo acids (2-oxoglutarate and oxaloacetate) at neutral pH. Oxaloacetate was undetectable (i.e. <0.05 nmol/mg of protein) at all stages of the incubation period. Results are the means for at least duplicate determinations, and extrapolations of rates are indicated by broken lines.

the flux through the pyruvate dehydrogenase complex by the addition of K⁺ (Nicklas *et al.*, 1971; Fig. 5). A similar effect can also be demonstrated by increasing the 3-hydroxybutyrate flux into the tricarboxylic acid-cycle (Table 1), suggesting that this phenomenon is associated with a generalized increase in carbon flux into the cycle rather than being specifically associated with a particular substrate.

Two possible mechanisms appear to be likely for this effect: (1) that intramitochondrial 2-oxoglutarate increases as a result of the stimulated tricarboxylic acid-cycle flux to an extent that glutamate transamination is inhibited; (2) that the increased production of C₂ units (acetyl-CoA) successfully competes for the oxaloacetate available, thus sequestering one component of the transamination reaction.

The first of these possibilities seems unlikely for a number of reasons. First, the production of 2-

oxoglutarate and aspartate seem to parallel each other, and thus after an increase in pyruvate flux a decrease in production of both 2-oxoglutarate and aspartate occurs (Fig. 5). Furthermore most of the accumulated 2-oxoglutarate is extramitochondrial, and exogenous 2-oxoglutarate is not a very effective inhibitor of glutamate transamination (Fig. 4). When glutamate transamination occurs in the presence of malate, 2-oxoglutarate accumulates extramitochondrially almost stoichiometrically with aspartate (Fig. 3), which suggests that malate is acting as the main source of oxaloacetate for transamination as well as permitting 2-oxoglutarate efflux from the mitochondria on the 2-oxoglutarate translocase (Brand & Chappell, 1974b). In fact, in the absence of malate, glutamate transamination by brain mitochondria in State-3 conditions is decreased 4-fold and 2-oxoglutarate accumulation is undetectable (S. C. Dennis & J. B. Clark, unpublished work).

Hence it seems more likely that the inhibitory effect of an increased acetyl-CoA metabolism on glutamate transamination lies in the competition of the glutamate-oxaloacetate transaminase and the citrate synthase for a common substrate, oxaloacetate. Under the conditions of the experiments reported in the present paper, oxaloacetate concentrations were always below the sensitivity of detection (<0.05 nmol/mg of protein) in spite of the presence of 2.5 or 5 mM-malate. This presumably is the result of the malate dehydrogenase equilibrium lying far in the direction of malate (Krebs, 1973). Furthermore the preferential utilization of the available oxaloacetate for citrate formation may be explained on the basis of the 20-fold lower apparent K_m for oxaloacetate of the citrate synthase enzyme in brain (2.5 μ M; Land & Clark, 1973) compared with the brain glutamate-oxaloacetate transaminase (K_m for oxaloacetate 50 μ M;

Dennis *et al.*, 1977). Additionally much of the intra-mitochondrial oxaloacetate may be protein-bound and may not be equally available to both enzymes, particularly if an enzyme-enzyme interaction between malate dehydrogenase and citrate synthase occurs (cf. Williamson *et al.*, 1972).

The physiological implications of the marked inhibition of glutamate transamination by acetyl-CoA metabolism by the citrate synthase reaction in brain mitochondria (Balazs, 1965; Nicklas *et al.*, 1971) and heart mitochondria (Durand *et al.*, 1969; Younes *et al.*, 1970; La Noue *et al.*, 1970) are of interest. Both these organs are highly differentiated towards specialized bioelectric phenomena, whereas tissues such as the liver, where the opposite occurs, i.e. pyruvate metabolism stimulates glutamate transamination (Haslam & Krebs, 1963), are organs with diverse metabolic activities. It has been proposed by Haslam & Krebs (1963) that the high glutamate-pyruvate transaminase activity in liver allows the use of an alternative 2-oxo acid (pyruvate) to oxaloacetate for glutamate transamination, thus avoiding the competition between the glutamate-oxaloacetate transaminase and citrate synthase observed in brain and heart.

The adult brain is also uniquely dependent on a rapid and continuous production of energy from glycolysis (Land *et al.*, 1977), unlike the liver, which may use different fuels according to circumstances. This necessitates the continual reoxidation of cytosolic NADH, which has been proposed to occur in many tissues by means of the malate-aspartate shuttle (Borst, 1963; Williamson *et al.*, 1971). Thus the process of glycolysis and pyruvate metabolism via the tricarboxylic acid cycle on the one hand and glutamate transamination and the Borst cycle on the other are closely integrated through their need for a

Table 2. Effect of metabolism of pyruvate and 3-hydroxybutyrate on extra-mitochondrial NADH oxidation by the malate-aspartate shuttle reconstituted with rat brain mitochondria

Mitochondria (1-1.5 mg of protein) were suspended in a final volume of 1 ml in the 100 mM-K⁺ medium (see the Methods section) containing (final concn.) 0.4 mM-NADH, 10 mM-glutamate, 2.5 mM-aspartate, 5 mM-malate, 10 μ g of malate dehydrogenase (12 units) and 2 mM-ADP to produce State-3 conditions (Chance & Williams, 1956). Where indicated, 5 mM-pyruvate or 10 mM-DL-3-hydroxybutyrate was also included. NADH oxidation was initiated by the addition of 20 μ g of glutamate-oxaloacetate transaminase (4 units) and was monitored in an Aminco Chance DW2 spectrophotometer in the dual-wavelength mode (340 nm - 373 nm) at 25°C. No reaction was observed unless both the added enzyme constituents of the Borst cycle were present. The results are expressed as nmol of NADH oxidized/min per mg of mitochondrial protein; the values in parentheses are the percentage inhibitions observed in the presence of pyruvate or 3-hydroxybutyrate.

Additions	NADH oxidation (nmol/min per mg of protein)							
	Non-synaptic mitochondria				Synaptic mitochondria			
	State 3		State 4		State 3		State 4	
None	55	(0)	15	(0)	28	(0)	9	(0)
10 mM-DL-3-Hydroxybutyrate	47	(15)	14	(7)	21	(25)	8	(11)
5 mM-Pyruvate	23	(60)	10	(33)	14	(50)	6	(33)

common intermediate, oxaloacetate. If for any reason a situation arises whereby pyruvate metabolism via the tricarboxylic acid cycle is restricted, then the Borst cycle may be stimulated to permit an increased rate of cytosolic NADH reoxidation, thus permitting glycolysis to continue and ATP concentrations to be maintained.

Table 2 shows the results of an experimental attempt to demonstrate this interrelationship between cytosolic NADH reoxidation via the Borst cycle and the carbox flux via the tricarboxylic acid-cycle. In these experiments the Borst cycle has been reconstituted by adding back to a brain mitochondrial preparation (either synaptic or non-synaptic) the cytosolic components of the Borst cycle, namely exogenous glutamate, aspartate, malate, malate dehydrogenase and glutamate-oxaloacetate transaminase. The oxidation of added exogenous NADH by the brain mitochondria only occurred in the presence of both enzyme preparations. If in addition either exogenous 3-hydroxybutyrate or pyruvate was added, the rate of exogenous NADH oxidation was markedly inhibited, thus demonstrating the inverse relationship that exists in brain mitochondria between the rate of carbon flux through the tricarboxylic acid-cycle and the rate of cytosolic NADH reoxidation. Such a closely integrated relationship between glycolysis, the tricarboxylic acid-cycle and the malate-aspartate shuttle is consistent with the brain's unique dependence on glycolysis for the maintenance of its bioelectric function.

S. C. D. is grateful to the Medical Research Council for personal finance and running expenses. We are grateful to Professor E. M. Crook for continued support and encouragement.

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