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GLUTAMATE METABOLISM IN RELATION TO GLUTAMATE TRANSPORT IN KIDNEY CORTEX MITOCHONDRIA OF RABBIT

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Summary

1. The metabolism of glutamate was followed by measurements of phospho*enol*pyruvate production, aspartate synthesis and ammonia release, whereas the transport of glutamate across the inner membrane of kidney cortex mitochondria was studied using an oxygen electrode and the swelling technique.

2. When added separately, avenaciolide and aminooxyacetate only partially inhibited both State 3 and uncoupled respiration of the mitochondria, as studied in the presence of glutamate as substrate. In contrast, the addition of both inhibitors to the reaction medium resulted in an almost complete inhibition of glutamate oxidation.

3. Swelling of kidney mitochondria in an isosmotic solution of ammonium glutamate was accelerated by uncoupler and inhibited by avenaciolide, while the swelling of mitochondria in potassium glutamate was stimulated by valinomycin and inhibited by uncoupler.

4. When glutamate was used as the sole substrate, inhibition of aspartate formation by aminooxyacetate resulted in a stimulation of both ammonia release and phospho*enol*pyruvate production. In contrast, with glutamate plus malate as substrate an elevation of the rate of glutamate deamination on the addition of aminooxyacetate was accompanied by an inhibition of phospho*enol*pyruvate synthesis in both State 3 and uncoupled conditions.

5. In the presence of valinomycin to induce K^+-permeability a marked enhancement of glutamate deamination was accompanied by a significant inhibition of glutamate transamination.

6. Based on the presented results it was concluded that in rabbit renal mitochondria utilizing glutamate as substrate the rates of ammonia production, phospho*enol*pyruvate formation and aspartate synthesis vary in response to different metabolic conditions, in which both the glutamate—H^+ symport and the glutamate—aspartate exchange systems are functioning to different extents.

Abbreviations: *P-enol*pyruvate, phospho*enol*pyruvate; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone.

Introduction

It is commonly accepted that in liver, glutamate is transported across the mitochondria membrane via both a glutamate—H^+ symport [1—4] and a glutamate—aspartate antiport system [1,4,5—8]. The same mechanisms of glutamate transport were postulated to exist in brain mitochondria [9,10], while heart mitochondria have been reported to possess only the glutamate—aspartate translocator [5,11].

Much of our knowledge concerning the transport of glutamate across the renal mitochondrial membrane has been derived from studies with rats [12—16] and pigs [14,17—19], which exhibit a high phosphate-dependent glutaminase activity [19—23]. In contrast, in the kidney cortex of both guinea pig and rabbit the glutaminase activity is much lower than that in rat kidney cortex [24], suggesting that in the former species the glutamate deamination could be of particular importance to maintain the acid—base homeostasis. However, a glutamate—H^+ symport system which would participate in the oxidation of glutamate by glutamate dehydrogenase [25] was rejected by Crompton and Chappell [18] on the basis of the unsatisfactory swelling behavior of kidney mitochondria. Moreover, Kovačević [14] proposed that the glutamate—aspartate antiporter translocating glutamate for the transamination pathway did not operate in pig-kidney mitochondria because of the complete inhibition of glutamate oxidation by avenaciolide, an inhibitor of the glutamate—H^+ symport system [26,27].

In the previous communication from this laboratory we reported that kidney mitochondria of rabbit readily utilize glutamate for the production of ammonia, phospho*enol*pyruvate and aspartate [28]. These observations suggest that rabbit kidney cortex mitochondria possess both glutamate—H^+ and glutamate—aspartate translocators. The aim of the present investigation was to study the glutamate metabolism in kidney cortex mitochondria of rabbit in relation to glutamate transport.

Materials and Methods

Mitochondrial preparation and incubation

Mitochondria were prepared from kidney cortex of white male rabbits (about 2—3 kg in weight) by a minor modification of the method of Schneider and Hogeboom [29] as described previously [28].

Oxygen consumption was measured polarographically using a Clark oxygen electrode. The standard incubation mixture contained: 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$, 40 mM Tris · HCl buffer, 10 mM potassium phosphate buffer, 10 mM potassium glutamate and about 2—3 mg of mitochondrial protein/ml. The final pH was 7.4. State 3 was induced by the following additions to the basic buffer: 20 mM glucose, 0.1 mM ADP and hexokinase (2—5 units/ml). Uncoupled mitochondria were produced by the addition of 0.1—0.2 μM FCCP. Reactions were started by the addition of mitochondrial suspension to the reaction medium. Mitochondrial incubations were performed in chambers maintained at 30°C. In metabolic studies oxygen was blown over the surface of the stirred reaction medium.

For measurements of the total content of metabolites in the reaction medium, 1 or 2-ml aliquots were removed at intervals as indicated in the legends to the tables. The protein was precipitated with 1/10 vol. of 35% (v/v) perchloric acid. The supernatant after centrifugation was neutralized with a mixture containing 5 M KOH and 0.5 M potassium acetate. $KClO_4$ was precipitated in the cold.

Swelling of mitochondria in isosmotic solutions of metabolites was followed by measuring the decrease of absorbance of mitochondrial suspensions at 520 nm on a Carl Zeiss Jena spectrophotometer.

Assays

Mitochondrial protein was determined by the biuret method [30], as described by Cleland and Slater [31]. Measurements of both phospho*enol*pyruvate and aspartate were done spectrophotometrically [32] while 2-oxoglutarate and malate were determined fluorimetrically [33]. Ammonia was assayed according to Chaney and Marbach [34] following the distillation of ammonia from the sample into sulphuric acid as described by Conway [35].

Enzymes and chemicals

Pyruvate kinase (EC 2.7.1.40) used for the phospho*enol*pyruvate assay was isolated and purified according to Tietz and Ochoa [36]. Malate dehydrogenase (EC 1.1.1.37) and aspartate aminotransferase (EC 2.6.1.1) were obtained by the methods of Englard and Siegel [37] and Sizer and Jenkins [38], respectively. $(NH_4)_2SO_4$-free hexokinase (EC 2.7.1.1) was purchased from Koch Light Laboratories Ltd., Colnbrook, England while lactate dehydrogenase (EC 1.1.1.27) was provided by Polskie Odczynniki Chemiczne, Gliwice, Poland. Valinomycin (Calbiochem, San Diego, Calif., U.S.A.) was kindly supplied by Professor L. Wojtczak and Dr. J. Popinigis. FCCP was the generous gift of Dr. P. Heytler while avenaciolide was kindly provided by Dr. E.J. Harris. All other chemicals were of analytical grade.

Results and Discussion

Oxygen electrode experiments

In agreement with a rather low glutaminase activity in rabbit kidney mitochondria [24] the uptake of oxygen with glutamine as sole substrate was very small (Table I). After the addition of malate the rate of oxygen consumption increased to the value similar to that for malate oxidation. In contrast, the rate of oxygen uptake in the presence of glutamate was about 6—7-fold higher in State 3 than that with glutamine. Like in liver mitochondria, uncoupler increased glutamate oxidation in renal mitochondria for about 50% in comparison to that measured in State 3. Valinomycin was effective as uncoupler stimulating the mitochondrial respiration (not shown).

As shown in Fig. 1 low concentrations (15—25 μM) of avenaciolide an inhibitor of the liver mitochondrial glutamate—H^+ symport [26,27], caused a significant decrease of glutamate oxidation in kidney cortex mitochondria of rabbit (for about 50%), while malate oxidation was not inhibited under these conditions (not shown). At higher (50 μM) avenaciolide concentrations the

TABLE I

EFFECT OF AVENACIOLIDE AND AMINOOXYACETATE ON THE RESPIRATION OF RABBIT KIDNEY CORTEX MITOCHONDRIA

Oxygen uptake was measured polarographically in either State 3 or uncoupled conditions as outlined under Materials and Methods, with approximately 1.5 mg of mitochondrial protein/ml. Glutamate, glutamine and malate were added at 10, 20 and 5 mM concentrations, respectively. Avenaciolide was added as an ethanolic solution to give a final concentration of 12.5 μM, and aminooxyacetate as the aqueous Tris salt to give a final concentration of 0.2 mM. All respiration rates are expressed as ngatoms of O/min per mg of protein. Values shown are means ± S.E. of 4 experiments.

Substrate	Aminooxyacetate	Avenaciolide	Respiration rate	
			State 3	Uncoupled
Glutamine	—	—	12.7 ± 2.8	11.5 ± 3.3
Glutamine + Malate	—	—	42.5 ± 3.9	19.7 ± 1.9
Malate	—	—	32.9 ± 3.1	19.7 ± 2.7
Glutamate	—	—	65.5 ± 3.1	88.0 ± 7.1
	+	—	36.5 ± 9.3	41.6 ± 3.4
	—	+	41.5 ± 4.1	44.0 ± 3.7
	+	+	5.7 ± 1.4	6.7 ± 1.4

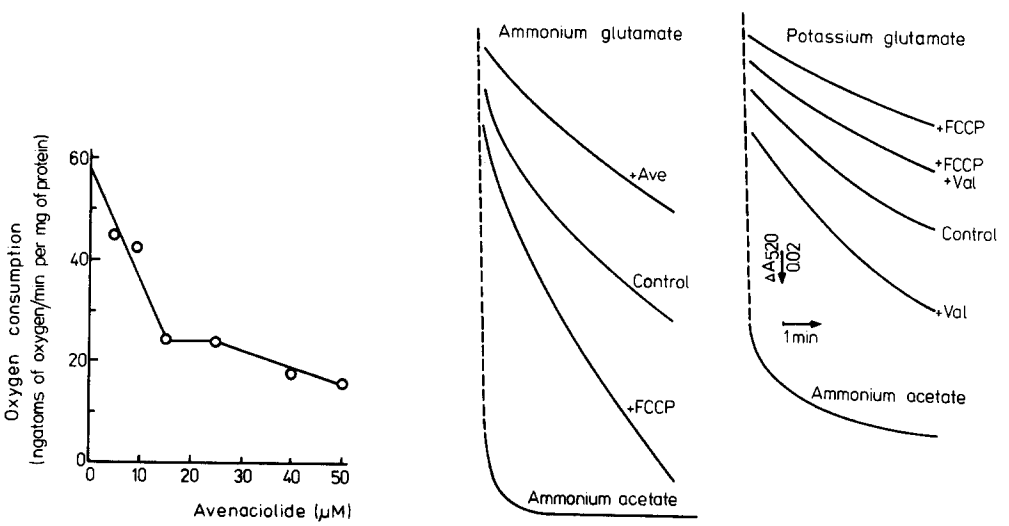

Fig. 1. Effect of avenaciolide on glutamate oxidation in rabbit kidney cortex mitochondria. The mitochondria (1.4 mg of protein/ml) were incubated for 3 min with avenaciolide before the addition of 10 mM glutamate. Oxygen uptake was measured polarographically in State 3 conditions, as outlined under Materials and Methods.

Fig. 2. Swelling of kidney cortex mitochondria of rabbit in isosmotic solutions of ammonium glutamate, potassium glutamate and ammonium acetate. The mitochondria (0.8 and 0.6 mg of protein in experiments, where ammonium glutamate and potassium glutamate were used, respectively) were suspended in medium containing: 10 mM Tris · HCl, 5 μM rotenone and either 100 mM ammonium glutamate or 100 mM potassium glutamate. The final pH was 7.4 and the final volume was 1.5 ml. The temperature was 30°C. Other additions were made at the following concentrations: 1.6 μM avenaciolide (Ave); 0.07 μM FCCP; 7 ng/ml valinomycin (Val). Swelling of mitochondria in 125 mM ammonium acetate, a known penetrant, is also presented for both experiments. The absorbance was recorded at 520 nm.

inhibition of glutamate oxidation was increased to about 80%. However, at this concentration of avenaciolide malate oxidation was also decreased (not shown). This agrees with the observation by McGivan and Chappell [26] that high concentrations of this substance inhibit respiration non-specifically. Avenaciolide at low concentrations was shown to inhibit completely glutamate oxidation in both pig and rat kidney cortex mitochondria [14,16].

Addition of aminooxyacetate, an inhibitor of aminotransferases [39] to rabbit renal mitochondria incubated with glutamate resulted in a significant decline in the rate of oxygen consumption under both conditions studied (Table I). Furthermore, an almost complete inhibition of glutamate oxidation was observed when both avenaciolide and aminooxyacetate were present in the reaction medium. These data suggest that rabbit renal mitochondria possess both a glutamate—H^+ symport and a glutamate—aspartate exchange system.

Swelling experiments

The glutamate—H^+ symport was examined by observing the rate of mitochondrial swelling in an isosmotic solution of ammonium glutamate [40]. The possibility of an electrogenic permeation of glutamate anion was evaluated in two ways: (i) by following the rate of swelling in isosmotic ammonium glutamate in the presence of FCCP, which allows electrogenic proton permeation [41] down the proton gradient created by the influx of NH_3 and (ii) by measuring the rate of swelling in isosmotic potassium glutamate in the presence of valinomycin to induce K^+-permeability [42].

In contrast to kidney cortex mitochondria of both rat [12,15] and pig [14,18] the rabbit renal mitochondria swelled significantly in ammonium glutamate (Fig. 2) and the swelling was decreased by avenaciolide suggesting the existence of a glutamate—OH^- antiport. However, in contrast to rat liver mitochondria [43] the rate of swelling of rabbit renal mitochondria in ammonium glutamate was stimulated in the presence of uncoupler which is indicative of electrogenic glutamate transport.

The swelling of rabbit renal mitochondria in potassium glutamate was increased by valinomycin and inhibited by uncoupler either in the presence or absence of valinomycin. This suggests the requirement of glutamate transport for potassium, shown by Meijer et al. [2], Harris et al. [44] and Debise et al. [4] for rat liver mitochondria. However, in contrast to rat liver mitochondria [43] the swelling of rabbit renal mitochondria in potassium glutamate (plus valinomycin) was not enhanced by omitting rotenone from the incubation medium (not shown). Thus it seems that a leak in the rotenone block is not responsible for the swelling of rabbit renal mitochondria in potassium glutamate (cf. ref. 2).

In view of these data the mechanism of glutamate transport in rabbit renal mitochondria is at present obscure. Mitochondria swell in ammonium glutamate and the swelling is inhibited by avenaciolide suggesting the existence of the glutamate—OH^- exchange. In contrast, the stimulation by uncoupler of mitochondrial swelling in ammonium glutamate as well as a significant swelling of mitochondria in potassium glutamate in the presence of valinomycin apparently mean that glutamate enters by a glutamate uniport. The existence of both these mechanisms was postulated by Kovačević [14] for glutamate

efflux from rat and pig mitochondria. The problem of the mechanism of glutamate influx into rabbit renal mitochondria needs further clarification.

Metabolic studies

Table II shows the rates of both phospho*enol*pyruvate and aspartate synthesis as well as those of ammonia release in kidney cortex mitochondria of rabbit incubated in State 3 and in the presence of uncoupler. The rates were linear up to 20 min of incubation under both conditions studied. As shown in Table II in rabbit renal mitochondria utilizing glutamate as sole substrate in State 3, very little phospho*enol*pyruvate and ammonia were formed, confirming our previous observation [28]. A high aspartate synthesis under these conditions seems to indicate that the glutamate transamination pathway [45] competes efficiently with phospho*enol*pyruvate carboxykinase for the intramitochondrial oxaloacetate. Malate production was negligible. In contrast to rat liver mitochondria [46,47] the rate of aspartate formation in uncoupled kidney cortex mitochondria of rabbit was not decreased below that measured in State 3, confirming our previous observation made for both rabbit liver [48] and guinea-pig liver mitochondria [49,50].

In uncoupled rabbit kidney mitochondria both ammonia release and phospho*enol*pyruvate production were about 2-fold higher than in State 3 probably due to a higher glutamate oxidation (cf. Table I). Addition of aminooxyacetate to mitochondria incubated in State 3 resulted in about a 2—3-fold increase in both ammonia release and phospho*enol*pyruvate production, while the stimulatory effect of aminooxyacetate on ammonia production in uncoupled mitochondria was smaller. An enhancement of phospho*enol*pyruvate synthesis in both State 3 and uncoupled mitochondria in the presence of aminooxyacetate could also be due to an increased flux through 2-oxoglutarate dehydrogenase, as indicated by a marked decline of 2-oxoglutarate levels in the incubation mixture (cf. Table III). These changes might result in a higher generation of both oxaloacetate and GTP for phospho*enol*pyruvate formation.

When malate was added to the reaction medium the rates of ammonia release

TABLE II

EFFECT OF AMINOOXYACETATE ON AMMONIA RELEASE, PHOSPHO*ENOL*PYRUVATE SYNTHESIS AND ASPARTATE PRODUCTION IN MITOCHONDRIA INCUBATED WITH EITHER GLUTAMATE OR GLUTAMATE PLUS MALATE IN STATE 3 AND IN UNCOUPLED CONDITIONS

Aminooxyacetate and malate were added at 0.2 and 5 mM concentrations, respectively. Other details as described under Materials and Methods. For measurements of the total content of metabolites in the reaction medium 2 ml aliquots were removed at 5-min intervals up to 20 min. Values are means ± S.E. of 4 experiments, given in nmol/min per mg of protein.

Incubation conditions	Aminooxyacetate	Glutamate			Glutamate plus malate		
		Ammonia	*P-enol*-pyruvate	Aspartate	Ammonia	*P-enol*-pyruvate	Aspartate
State 3	—	4.8 ± 0.3	2.3 ± 0.4	18.9 ± 2.1	1.0 ± 0.3	18.1 ± 1.5	40.0 ± 5.2
	+	9.5 ± 1.3	6.6 ± 0.5	—	9.6 ± 0.8	8.4 ± 1.1	—
Uncoupled	—	8.2 ± 1.9	3.8 ± 0.5	17.3 ± 2.9	0.9 ± 0.4	20.0 ± 2.1	35.1 ± 3.1
	+	9.9 ± 0.3	6.6 ± 0.9	—	8.0 ± 0.9	6.1 ± 1.0	—

TABLE III

EFFECT OF AMINOOXYACETATE ON THE ACCUMULATION OF 2-OXOGLUTARATE IN THE MITOCHONDRIAL SUSPENSION INCUBATED IN STATE 3 AND IN UNCOUPLED CONDITIONS

Experimental conditions as in Table II. The level of 2-oxoglutarate was determined after 15 min of incubation of mitochondria (2.6 mg protein/ml) in the reaction medium. The values are given in nmol/mg of protein. Assays were performed in duplicate.

Substrate	Aminooxyacetate	2-Oxoglutarate	
		State 3	Uncoupled
Glutamate	—	2.7	3.4
	+	0.5	1.0
Glutamate + malate	—	54.4	54.2
	+	0.6	0.6

were negligible under both State 3 and uncoupled conditions. A 2-fold higher aspartate production than that measured with glutamate as a sole substrate indicates that in the presence of malate glutamate transamination is increased due to a higher oxaloacetate generation from malate. A high phospho*enol*pyruvate formation under these conditions is also probably due to an increased oxaloacetate generation from malate. The same order of phospho*enol*pyruvate synthesis in uncoupled mitochondria as that in State 3 suggests that, as in guinea-pig liver mitochondria, the provision of GTP by substrate level phosphorylation is also essential to support phospho*enol*pyruvate formation in rabbit kidney cortex mitochondria [51].

Although the addition of aminooxyacetate resulted in a marked increase in ammonia production the phospho*enol*pyruvate formation decreased about 2—3-fold under both State 3 and uncoupled conditions, reaching the levels measured in the absence of malate in the reaction medium. These observations suggest that the glutamate—H^+ symport and/or glutamate deamination could limit phospho*enol*pyruvate synthesis probably due to a lower 2-oxoglutarate generation in comparison to that when the transamination pathway is operating. This could result in providing less substrate for GTP production. Concentrations of 2-oxoglutarate measured in the presence and absence of aminooxyacetate under the conditions studied confirm this suggestion (cf. Table III).

Addition of leucine, an activator of glutamate dehydrogenase [52,53], to rabbit renal mitochondria incubated with glutamate in State 3 resulted in about a 2-fold increase in both ammonia release and phospho*enol*pyruvate formation (Table IV), confirming our previous observation for uncoupled mitochondria [28]. The level of 2-oxoglutarate was low and practically unchanged on addition of leucine while the malate concentration was slightly increased in the presence of either leucine or aminooxyacetate in the reaction medium. When glutamate plus malate was used as substrate, leucine stimulated neither ammonia release nor phospho*enol*pyruvate formation. Rates of both ammonia production and phospho*enol*pyruvate synthesis as well as levels of 2-oxoglutarate following the addition of aminooxyacetate were similar to those presented in Tables II and III, respectively.

In order to determine whether glutamate metabolism is dependent upon

TABLE IV

EFFECT OF AMINOOXYACETATE AND LEUCINE ON AMMONIA RELEASE, PHOSPHO*ENOL*-PYRUVATE SYNTHESIS, ASPARTATE FORMATION AND ACCUMULATION OF 2-OXOGLUTARATE AND MALATE IN THE MITOCHONDRIAL SUSPENSION INCUBATED WITH EITHER GLUTAMATE OR GLUTAMATE PLUS MALATE IN EITHER STATE 3 OR IN THE PRESENCE OF VALINOMYCIN

The mitochondrial suspension was incubated for 10 min either in State 3 or in the presence of valinomycin (1 μg/ml) in the reaction medium described under Materials and Methods. Malate, aminooxyacetate and L-leucine were added at 5, 0.2 and 2.5 mM concentrations, respectively, where indicated. For measurements of ammonia, *P-enol*pyruvate and aspartate samples were removed from the incubation mixture at 2-min intervals, while the accumulation of both 2-oxoglutarate and malate was determined in duplicate after 10 min of incubation. Values expressing the rates of ammonia release, *P-enol*pyruvate synthesis and aspartate production are means ± S.E. of 3 experiments.

Conditions	Substrate	Additions	Ammonia *	*P-enol*-pyruvate *	Aspartate *	2-Oxo-gluta-rate **	Malate **
State 3	Glutamate	None	5.0 ± 0.8	2.9 ± 0.5	18.9 ± 2.1	3.9	1.8
		Aminooxy-acetate	9.5 ± 1.3	6.6 ± 0.5	—	1.7	2.5
		Leucine	9.0 ± 0.7	5.5 ± 0.7	21.3 ± 2.9	3.3	3.0
	Glutamate plus malate	None	1.0 ± 0.3	18.1 ± 1.5	40.0 ± 5.2	38.7	—
		Aminooxy-acetate	9.6 ± 0.8	8.4 ± 1.1	—	1.7	—
		Leucine	1.5 ± 0.3	18.0 ± 1.2	38.2 ± 4.3	45.0	—
With Valinomycin	Glutamate	None	8.9 ± 0.9	6.1 ± 1.2	6.1 ± 1.7	6.8	9.1
		Aminooxy-acetate	14.4 ± 0.7	7.2 ± 0.8	—	0.1	17.7
		Leucine	18.9 ± 0.6	8.2 ± 1.9	5.3 ± 1.4	7.6	27.2
	Glutamate plus malate	None	8.7 ± 0.9	11.2 ± 2.6	7.5 ± 0.8	15.2	—
		Aminooxy-acetate	8.4 ± 0.5	7.1 ± 1.1	—	0.2	—
		Leucine	8.7 ± 0.7	8.5 ± 0.8	7.4 ± 0.1	18.4	—

* Results in nmol/min per mg protein.

** Results in nmol per mg protein.

an electrical potential gradient across the mitochondrial membrane we have studied the effect of valinomycin on glutamate metabolism since high concentrations of K^+ in the presence of this antibiotic diminish the size of the transmembrane ($\Delta\psi$) gradient [54]. In the presence of valinomycin, the rates of ammonia release, phospho*enol*pyruvate formation and aspartate synthesis in rabbit renal mitochondria were linear with time up to 10 min of incubation. Thus, incubations of mitochondria were carried out only for 10 min. On the addition of valinomycin to renal mitochondria incubated with either glutamate or glutamate plus malate as substrate, the rate of aspartate production was markedly decreased (Table IV). This could be due to an inhibition of aspartate transport across the mitochondrial membrane [5] resulting from a decrease of the membrane potential in the presence of K^+ and valinomycin [54].

On the addition of valinomycin to mitochondria incubated with glutamate as the sole substrate the rate of ammonia release was as high as in the uncoupled mitochondria (cf. Tables II, and IV). In State 3, addition of aminooxyacetate to inhibit completely the glutamate—aspartate translocator resulted in about a

2-fold elevation of ammonia formation as did addition of leucine, an activator of glutamate dehydrogenase [52,53]. However, in contrast to State 3 the addition of either aminooxyacetate or leucine to mitochondria incubated with valinomycin and glutamate did not practically change the rate of phospho*enol*-pyruvate production. Since in the presence of either leucine or aminooxyacetate a significant increase in malate accumulation was observed, the fact that there is no further stimulation of phospho*enol*pyruvate synthesis by either aminooxyacetate or leucine could be due to an energy-dependent uptake of K^+, resulting in a decrease of energy available for phospho*enol*pyruvate production. This is made possible in terms of GTP metabolism due to the conversion of GTP to ATP by nucleotide diphosphate kinase being kinetically favored over the reverse direction because of the inhibitory affects of Mg-ADP [55,56] which probably increases in the presence of valinomycin due to its uncoupling action [57].

When valinomycin-treated mitochondria were incubated with glutamate plus malate neither aminooxyacetate nor leucine increased the rate of ammonia release or of phospho*enol*pyruvate formation. This could be due to an increase in the level of malate and some other dicarboxylic acids in the mitochondria preventing the activatory effects of ADP and leucine on the glutamate dehydrogenase since anions are known to abolish the regulatory influence of positive modulators on the activity of this enzyme [58,59].

Based on the presented results it is possible to conclude that in rabbit renal mitochondria utilizing glutamate as substrate the rates of ammonia production, phospho*enol*pyruvate formation and aspartate synthesis vary in response to different metabolic conditions, in which both the glutamate—H^+ symport and the glutamate—aspartate exchange systems are functionning to a different extent. In both State 3 and uncoupled mitochondria oxidizing glutamate plus malate, glutamate is utilized for aspartate synthesis, while glutamate deamination practically does not occur (cf. Table II) suggesting that under these conditions glutamate is transported across the inner membrane primarily via the glutamate—aspartate exchange. In mitochondria incubated with glutamate as a sole substrate in either State 3 or with uncoupler, relatively high rates of aspartate production and ammonia release (cf. Table II) indicate that both systems are involved in the influx of glutamate. In the presence of valinomycin to induce K^+-permeability [42] in mitochondria utilizing glutamate with or without malate, glutamate deamination is as high as in uncoupled mitochondria while the rate of glutamate transamination is significantly decreased (cf. Table IV) suggesting that in the presence of valinomycin the transport of glutamate via the glutamate—H^+ symport appears to operate more actively than the glutamate—aspartate exchange. Under all conditions studied the rate of phospho*enol*pyruvate synthesis from either glutamate or glutamate plus malate is dependent upon the provision of GTP from substrate level phosphorylation.

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