INHIBITION OF PHOSPHOENOLPYRUVATE TRANSPORT VIA THE TRICARBOXYLATE AND ADENINE NUCLEOTIDE CARRIER SYSTEMS OF RAT LIVER MITOCHONDRIA

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Received June 4, 1973

SUMMARY

The translocation of phosphoenolpyruvate by the tricarboxylate carrier system in rat liver mitochondria was shown to be inhibited by atractyloside and long chain fatty acyl CoA esters as well as benzene, 1, 2, 3 tricarboxylate. By contrast benzene 1, 2, 3 tricarboxylate did not inhibit atractyloside sensitive adenine nucleotide translocation catalyzed by phosphoenolpyruvate. These results indicate that although phosphoenolpyruvate is preferentially transported by the tricarboxylate carrier system, it may also be transported by the adenine nucleotide translocase. The inhibition of the adenine nucleotide and tricarboxylate carrier systems by atractyloside and long chain acyl CoA esters indicates a close functional interrelationship of these transport carriers in the inner mitochondrial membrane. Moreover, the potent inhibition of phosphoenolpyruvate, citrate, and adenine nucleotide transport by long chain acyl CoA's provides further evidence that these esters are natural effectors which participate in the regulation of gluconeogenesis, lipogenesis, and energy-linked respiration.

INTRODUCTION

Previous reports from this laboratory (1, 2, 3) dealing with the inhibition of the adenine nucleotide translocase system have provided evidence for the role of long chain fatty acyl CoA esters in the regulation of mitochondrial metabolism. In a related study (4), it was shown that citrate and malate transport on the tricarboxylate carrier were inhibited by a relatively low concentration of palmitoyl CoA. Other investigations have established that phosphoenolpyruvate (PEP) may be transported across the mitochondrial membrane by the tricarboxylate carrier (5), and that this transport system appears to be rather nonspecific (6). Whereas, it has generally been considered that the adenine nucleotide translocase was very specific for ADP and ATP (7), recent investigations concerned with the

inhibitory effect of PEP on mitochondrial protein synthesis (8, 9), suggested that PEP may also be transported by the adenine nucleotide translocase system. The present investigation shows that PEP, although preferentially exchanged on the tricarboxylate carrier system, may also be transported by the adenine nucleotide translocase. It will be shown that although only the tricarboxylate system was sensitive to inhibitory concentrations of benzene 1, 2, 3 tricarboxylate (1, 2, 3 BTCA), both anion transport systems were inhibited by atractyloside and by very low levels of long chain acyl CoA esters. The exceptionally potent inhibitory property of long chain acyl CoA esters towards the transport of PEP across the mitochondrial membrane offers a possible explanation for the inhibition by fatty acids of gluconeogenesis in the liver of animals capable of synthesizing large quantities of PEP in the mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria were prepared by the method of Johnson and Lardy (10), except that a medium of 250 mM sucrose, 4 mM Tris-Cl, and 1 mM Tris-EGTA (final pH 7.4) was used throughout the preparation, and also for the final suspension of mitochondria. Oxygen consumption was determined on a Gilson oxygraph using a Clark electrode, and respiratory control ratios were calculated for all mitochondria used according to Estabrook (11).

Adenine nucleotide translocase activity and uptake of (^{14}C)-PEP were measured both by the forward exchange as previously described (2), and the back-exchange techique of Pfaff and Klingenberg (12). Loading of mitochondria with (^{14}C)-citrate was carried out according to the following procedure: approximately 200 mg of mitochondrial protein was added to 4 ml of a mixture which contained (final concentration) 200 mM mannitol, 50 mM Tris-HCl (pH 7.4), 2 μg/ml rotenone, 0.5 μg/ml antimycin A and 0.5 to 1.0 μ Ci of the (^{14}C)-

TABLE 1

The effect of oleoyl CoA, atractyloside, and 1, 2, 3 BTCA on the exchange of intramitochondrial (^{14}C)-ATP with various extramitochondrial anions. Mitochondria were loaded with (^{14}C)-ATP (8,756 DPM) and incubated as described in materials and methods. The exchange reaction was carried out with 3.4 mg mitochondrial protein per incubation at 30° and terminated after 4 minutes by the addition of 5 mM atractyloside. The concentration of inhibitors tested was 5 μM oleoyl CoA, 5 mM atractyloside and 50 mM 1, 2, 3 BTCA. The results are the mean values obtained in duplicate incubations from a representative experiment.

		Percentage Exchange Activity			
ANION	CONC (mM)	CONTROL	OLEOYL CoA	ATRACTYLOSIDE	1,2,3 BTCA
PEP	1.0	18.2	2.2	1.6	15.6
	5.0	67.9	5.3	3.4	71.9
ADP	0.1	82.8	8.6	3.3	80.8
ATP	0.1	71.3	3.4	2.1	73.2
CITRATE	10	2.8	1.3	-	-
MALATE	10	2.3	1.8	-	-
PYRUVATE	10	1.9	1.3	-	-

labeled substrate. The loading was performed at 0° for 4 minutes. The suspension was then centrifuged for 10 minutes at 18,000 g at 5° in a refrigerated Sorvall centrifuge. This loading procedure is essentially the same as that described by Kleineke, et al.(6). The exchange reactions, determination of radioactivity, and calculation of the percentage of exchange were made as described by Kleineke et al. (6). (1-^{14}C)-PEP was customly prepared by the New England Nuclear Co., (1,5-^{14}C)-citrate, and (^{14}C)-ATP were purchased from Amersham-Searle; acyl CoA esters from P-L Biochemicals, 1, 2, 3 BTCA from K & K Lab Inc. and atractyloside from Calbiochem. All other reagents were of the highest grades commercially available.

RESULTS AND DISCUSSION

Mitochondria were loaded with (^{14}C)-ATP, and the back-exchange

TABLE II

The inhibition of the (^{14}C)-citrate exchange with various anions. Mitochondria were loaded with (^{14}C)-citrate (83,179 DPM) as described in Materials and Methods. Mitochondrial protein was 6.3 mg per incubation and the concentration of inhibitors was the same as in Table 1. The temperature of the exchange reaction was 4°, and after 2 min incubation, mitochondria were separated by centrifugation at 18,000 g for 10 min. The results are mean values obtained in duplicate incubations from a representative experiment. Negative values indicate that in the presence of that anion the counts in the supernatant were lower than when incubated in buffer only (5).

		Percentage Exchange Activity			
ANION	CONC (mM)	CONTROL	OLEOYL CoA	ATRACTYLOSIDE	1,2,3 BT(
CITRATE	1	71.9	-2.6	-11.1	-0.5
PEP	1	64.4	-1.1	-8.3	-1.2
ADP	1	0.3	-	-	-
	10	1.7	-	-	-
ATP	1	0.9	-	-	-
	10	2.4	-	-	-
MALATE	1	31.4	-2.2	-6.7	0.3
PYRUVATE	1	0.3	-	-	-

technique of Pfaff and Klingenberg (12) was used to assay the adenine translocase activity. Table 1 shows that at relatively high concentrations, PEP caused a rapid and atractyloside sensitive efflux of (^{14}C)-ATP. This is in agreement with a previous study of McCoy and Doeg (9). It is interesting to note that while ADP and ATP are effective in catalyzing this exchange with PEP; citrate, malate, and pyruvate are completely without effect. These results suggest that of the anions transported by the tricarboxylate carrier, apparently only PEP may also be transported across the inner mitochondrial membrane by the adenine nucleotide translocase system. This idea is further supported by the fact that while the PEP/(^{14}C)-ATP exchange

was abolished by atractyloside, the reaction was insensitive to concentrations of 1, 2, 3 BTCA that completely inhibit the tricarboxylate carrier. The ability of PEP to catalyze the efflux of intramitochondrial ATP was temperature dependent. When the experiment was carried out at 10° the percent exchange was approximately one half of that depicted in Table 1. The potent inhibition of the adenine nucleotide translocase system by oleoyl CoA shown in Table 1 was observed for other long chain acyl CoA esters, i.e. palmitoyl and myristoyl CoA.

Table II summarizes the results of experiments in which the exchange of intramitochondrial (^{14}C)-citrate on the tricarboxylate carrier system was tested with a number of anions. As previously shown (13, 6) 1, 2, 3 BTCA specifically inhibits citrate, malate and PEP transport on the tricarboxylate carrier. The transport inhibition pattern of these three substrates by atractyloside and acyl CoA suggests a possible functional relationship between the tricarboxylate and adenine nucleotide carriers. The resemblance of atractyloside to the adenine moiety (14) and the presence of ADP in the acyl CoA molecule can account for the specific inhibition of adenine nucleotide translocase by these compounds (15); however, their interaction with the tricarboxylate carrier system is less clear.

The uptake of (^{14}C)-PEP by mitochondria is shown in Table III. The addition of ADP inhibited the uptake by 20%. 1, 2, 3 BTCA which blocks the transport of PEP by the tricarboxylate carrier reduced the uptake by 50%. The addition of ADP together with 1, 2, 3 BTCA caused a further diminution of counts (71% of control) indicating that the ADP was competing with PEP for transport by the adenine nucleotide translocase.

The inhibition of the tricarboxylate and adenine nucleotide translocase systems by atractyloside and acyl CoA's demonstrates a previously unsuspected biochemical relationship between these two

TABLE III

The effect of ADP on the uptake of (^{14}C)-PEP into mitochondria. Incubation conditions for the uptake of (^{14}C)-PEP (118,230 DPM) were as described for the forward adenine nucleotide translocase assay under Materials and Methods. 2.3 mg mitochondrial protein was incubated at 4° for 2 minutes and separated by centrifugation at 18,000 xg for 10 minutes. The concentration of ADP was 0.1 mM and 1, 2, 3 BTCA 50 mM. The results are mean values obtained in duplicate incubations from a representative experiment.

ADDITIONS	DPM/PELLET	% INHIBITION
None	33,700	0
ADP	25,193	25
1, 2, 3 BTCA	15,765	53
ADP + 1, 2, 3 BTCA	9,813	71

carriers. Furthermore, as suggested by a number of studies from this (2) and other laboratories (14, 16), physiologically important compounds such as, long chain acyl CoA esters which can effectively control the mitochondrial transport of PEP, citrate, and adenine nucleotides must play a central role in cellular metabolism. The inhibition of the efflux of PEP from mitochondria by acyl CoA esters may partially explain the postulated inhibition of gluconeogenesis in guinea pig liver by fatty acids (17, 18). Inhibition of citrate efflux by long chain acyl CoA esters could account for low rates of lipogenesis during starvation as suggested by Halperin *et al.* (4).

ACKNOWLEDGEMENTS

This research was supported by NIH Grant GM 14033. We wish to acknowledge the expert technical assistance of Mrs. Cheryll Crosby and thank Dr. Henry Lardy for his continued interest and encouragement.

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