α-Oxoglutarate Carboxylation in Liver Mitochondria from Normal, Alloxan Diabetic and Streptozotocin Diabetic Rats (36068)

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Several published reports have suggested that the fixation of CO_2 by NADP-linked isocitrate dehydrogenase may represent an important metabolic step in the synthesis of tricarboxylic acid cycle intermediates (1–4). Wagle (3) has shown that the "α-oxoglutarate carboxylase" activity of liver mitochondria from alloxan diabetic rats was greatly increased when assayed by the method of Ochoa (2). This finding led to the suggestion that α-oxoglutarate carboxylation is an important pathway for supporting the increased rate of gluconeogenesis present in alloxan diabetes (4). In the present study, we have examined α-oxoglutarate carboxylation by intact rat liver mitochondria from normal and diabetic rats and have observed the effects of *in vitro* administration of insulin, glucagon, dibutyryl cyclic AMP and octanoylcarnitine on this carboxylation.

Methods. Male Sprague-Dawley rats (weighing approx 150 g) were fasted for 48 hr and made diabetic by administration of either alloxan (200 mg/kg, sc) or streptozotocin (75 mg/kg, iv). Purina Lab Chow was fed *ad libitum* until the animals were killed 30 days later. No attempt was made to increase rat survival by the administration of insulin. Mortality was quite high; 65% of the alloxan diabetic and 10% of the streptozotocin diabetic rats died during the 30 day period. Of the remaining diabetic animals, only those with blood glucose levels above 300 mg/100 ml were used for the experiments in this study.

Mitochondria were isolated by the method of Schneider (5) as modified by Johnson and Lardy (6), and suspended in 0.25 *M* sucrose at a concentration of 30 mg of protein/ml. Mitochondrial incubations were carried out in stoppered 25 ml Erlenmeyer flasks which were shaken in a water bath at 37°. Reactions were started by addition of 0.5 ml of mitochondrial suspension to 2.5 ml of media; and were stopped by addition of 3 ml of perchloric acid. Processing of samples for analysis was done by the method of Walter *et al.* (7). The compositions of the incubation media are given in the legends to Tables I and II.

TABLE I. α-Oxoglutarate Consumption and Metabolic Formation by Normal Rat Liver Mitochondria.[a]

Incubation time (min)	α-oxoglutarate consumed	Accumulated (μmoles/10 mg of protein)			
		Malate	Fumarate	Succinate	Citrate
5	1.2	.71	0.2	0	0.24
10	2.3	1.29	0.3	0.1	0.46
20	4.6	2.70	0.6	0.2	0.96
30	6.9	4.00	0.9	0.3	1.35
40	8.9	5.15	1.2	0.4	1.65

[a] The 3 ml reaction mixtures (pH 7.2) contained 4 m*M* ATP, 10 m*M* $MgCl_2$, 6.7 m*M* P_i, 13.3 m*M* KTEA, 6.7 m*M* α-oxoglutarate, and 13.3 m*M* HCO_3^-. Mitochondria equal to 10 mg of protein were added at time 0; and the incubation times were varied as indicated.

TABLE II. Effects of Streptozotocin and Alloxan Diabetes on α-Oxoglutarate Metabolism in Rat Liver Mitochondria.[a]

Treatment	No. of rats	Metabolite changes (nmoles/20 min/mg of protein ± SEM) α-Oxoglutarate consumed	Citrate accumulated
Normal	10	631 ± 38	91.8 ± 3.8
Streptozotocin diabetic	8	831 ± 81[b]	132 ± 9[c]
Alloxan diabetic	4	894 ± 85[d]	141 ± 14[c]

[a] The 3 ml reaction mixtures (pH 7.4) contained 4 m*M* ATP, 6.7 m*M* P_i, 13.3 m*M* KTEA, 2 m*M* EDTA, 10 m*M* $MgCl_2$, 6.7 m*M* α-oxoglutarate and 20 m*M* HCO_3^-. The incubation flasks were gassed with a mixture of 95% O_2 + 5% CO_2.

[b] $p < .05$.

[c] $p < .001$.

[d] $p < .025$.

Results and Discussion. Rat liver mitochondria, incubated in a medium containing bicarbonate, converted α-oxoglutarate to malate and fumarate via oxidation and to citrate via carboxylation (Table I). Under these experimental conditions, citrate is not formed via citrate synthase, even in the presence of fatty acids, because the high $NADH_2$:NAD ratio, which prevails in the absence of a phosphate acceptor, prevents oxidation of malate to oxalacetate (8). In addition, the citrate, which is formed via α-oxoglutarate carboxylation, is not lost by cleavage to oxalacetate and acetate or acetyl-CoA (8). Thus, citrate accumulation represented only α-oxoglutarate carboxylation.

The effects of diabetes on α-oxoglutarate metabolism are shown in Table II. Mitochondria from both the streptozotocin and alloxan diabetic rats showed significant increases in both α-oxoglutarate consumption and citrate accumulation. α-Oxoglutarate consumption increased at a rate twice that of citrate accumulation in accord with the dismutation:

2 α-oxoglutarate → succinate + citrate.

Our results with intact mitochondria complement those of Wagle (3) which showed that liver mitochondrial "α-oxoglutarate carboxylase" (NADP-isocitrate dehydrogenase) activity was increased by alloxan diabetes. However, the physiological importance of mitochondrial α-oxoglutarate carboxylation and, therefore, its function in diabetes still remains obscure.

L-Octanoylcarnitine was added to mitochondria from normal and streptozotocin diabetic rats to determine if fatty acid oxidation, which facilitates gluconeogenesis, could increase the rate of α-oxoglutarate carboxylation (Table III). The oxidation of octanoylcarnitine was primarily to β-hydroxybutyrate, thus resulting in a very high β-hydroxybutyrate to acetoacetate ratio in both normal (18 ± 2) and diabetic (16 ± 1) mitochondria. From the equilibrium constant of β-hydroxybutyrate dehydrogenase (9), these ratios gave $NADH_2$:NAD ratios of 0.93 ± 0.12 and 0.80 ± 0.08, respectively. These very reduced conditions did not, however, lead to increased accumulations of citrate. These data support our previous conclusion with normal mitochondria that α-oxoglutarate carboxylation is driven at maximal rate by α-oxoglutarate oxidation alone and is, therefore, not increased by the presence of other oxidizable substrates (8). However, if α-oxoglutarate oxidation is retarded, *e.g.*, by the addition of inhibitors such as arsenite, other substrates can contribute reducing equivalents (8). Since the rate of α-oxoglutarate carboxylation was maximal in these experiments (Tables II and III), expression of the data on a per gram of liver basis gives an estimation of the maximum contribution of α-oxoglutarate carboxylation to gluconeogenesis. Assuming that liver contains 8.3 mg

TABLE III. Effects of Octanoylcarnitine on α-Oxoglutarate Metabolism by Mitochondria from Normal and Streptozotocin Diabetic Rats.[a]

Treatment	No. of rats	Additions (m*M*)	Metabolite changes (nmoles/20 min/mg of protein ± SEM)				
			α-Oxoglutarate consumed	Citrate accumulated	β-Hydroxybutyrate accumulated	Acetoacetate accumulated	β-Hydroxybutyrate/acetoacetate
Normal	5	BSA[b] (0.15)	522 ± 11	101 ± 4	—	—	—
		BSA (0.15) + L-octanoylcarnitine (1.0)	319 ± 14	90 ± 3	256 ± 7	15 ± 1	18 ± 2
Diabetic	5	BSA (0.15)	602 ± 20	125 ± 4	—	—	—
		BSA (0.15) + L-octanoylcarnitine (1.0)	366 ± 19	112 ± 4	294 ± 14	19 ± 1	16 ± 1

[a] The reaction mixtures were the same as described for Table II.
[b] Bovine serum albumin.

of mitochondrial nitrogen per gram of tissue (7), which is approximately 51.9 mg of mitochondrial protein per gram, maximal citrate synthesis via α-oxoglutarate carboxylation corresponds to 0.24 ± 0.01 μmoles/min/g of liver in the normal rats; 0.34 ± 0.02 in the streptozotocin diabetic rats, and 0.36 ± 0.04 in the alloxan diabetic rats.

Additions of dibutyrul cyclic AMP (0.5 m*M*), insulin (1.3 units/ml), and glucagon (0.01 m*M*) to the incubation media did not affect α-oxoglutarate metabolism in mitochondria from normal or diabetic rats. This indicates that the dismutation α-oxoglutarate oxidation–carboxylation is probably not subject to regulation, on the mitochondrial level of organization, by the direct actions of these substances.

Summary. Rat liver mitochondria, incubated in media containing 4 m*M* ATP, 10 m*M* $MgCl_2$, 6.7 m*M* P_i, 13.3 m*M* TEA, 6.7 m*M* α-oxoglutarate, and 20 m*M* HCO_3^-, oxidized α-oxoglutarate through the tricarboxylic acid cycle and utilized a portion of the reducing equivalents produced by this oxidation to drive α-oxoglutarate to citrate via reductive carboxylation. Streptozotocin and alloxan diabetes increased the rates of oxidation and citrate accumulation. L-Octanoylcarnitine inhibited both α-oxoglutarate oxidation and carboxylation. Insulin, glucagon, and dibutyryl cyclic AMP, added *in vitro*, did not affect α-oxoglutarate oxidation or carboxylation.

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1. Ochoa, S., J. Biol. Chem. **159**, 143 (1945).
2. Ochoa, S., J. Biol. Chem. **174**, 133 (1948).
3. Wagle, S. R., Biochim. Biophys. Acta **97**, 142 (1965).
4. Wagle, S. R., Diabetes **15**, 19 (1966).
5. Schneider, W. C., J. Biol. Chem. **176**, 259 (1948).
6. Johnson, D., and Lardy, H. A., Methods Enzymol **10**, 94 (1967).
7. Walter, P., Paetkau, V., and Lardy, H. A., J. Biol. Chem. **241**, 2523 (1966).
8. Mackerer, C. R., and Mehlman, M. A., *in* "Symposium on Metabolic Regulation: Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria" (M. A. Mehlman and R. W.

Hanson, eds.), Academic Press, New York (1971), in press.

9. Krebs, H. A., and Veech, R. L., *in* "The Energy Level and Metabolic Control in Mitochondria" (S. Papa, J. M. Tager, E. Quagliariello, and E. C. Slater, eds.), p. 329. Adriatica Editrice, Bari, Italy (1969).

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