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THE INHIBITION OF L(—)-PALMITYLCARNITINE OXIDATION BY α-KETOGLUTARATE IN RAT LIVER MITOCHONDRIA

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SUMMARY

1. The effect of α-ketoglutarate addition to uncoupled rat liver mitochondria oxidizing L(—)-palmitylcarnitine *plus* L-malate was studied with regard to various metabolic parameters.

2. L(—)-Palmitylcarnitine *plus* L-malate oxidation was strongly inhibited by α-ketoglutarate in uncoupled rat liver mitochondria. This inhibition was dependent upon the concentration of α-ketoglutarate.

3. Citrate production was markedly inhibited following α-ketoglutarate addition but ketogenesis was not stimulated. The inhibition of citrate production was not correlated with a high ATP level in the mitochondrial suspension but followed closely the inhibition of oxygen consumption.

4. ADP addition prevented the establishment of the α-ketoglutarate induced inhibitory state. The prevention of the inhibitory state by ADP addition was atractyloside sensitive but oligomycin insensitive.

5. The inhibitory state induced by α-ketoglutarate oxidation was not specific for L(—)-palmitylcarnitine *plus* L-malate oxidation but caused strong inhibition of the oxidation of all NAD-linked substrates. Succinate oxidation was not affected.

6. It is postulated that this inhibitory effect induced by α-ketoglutarate was due to an elevated intramitochondrial level of GTP which inhibited NAD-linked substrate oxidation.

INTRODUCTION

Liver mitochondria have the metabolic capability to convert acetyl-CoA either to acetoacetate in the process of ketogenesis or to carbon dioxide in the oxidative reactions of the citric acid cycle. Experimental consideration of the regulation of the fate of acetyl-CoA has centered primarily on the regulation of the citrate synthase reaction of the mitochondrion which is essential for complete oxidation of two-carbon units in the citric acid cycle.

It has been demonstrated[1-3] that physiological concentrations of ATP inhibit purified citrate synthase (citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7) and, as a result, it was postulated that the energy level of the mitochondrion may

Abbreviation: FCCP, *p*-trifluoromethoxyphenylhydrazone of carbonyl cyanide.

determine whether acetyl units enter the citric acid cycle or are converted to ketone bodies[4–6]. On the other hand, it was shown that significant alternations of the intramitochondrial ATP level have no direct effect on the relative rates of ketogenesis or citrate formation in isolated rat liver mitochondria[7,8]. Various laboratories have concluded that the availability of the substrate oxalacetate for the citrate synthase reaction may be the primary determinant of the fate of acetyl-CoA derived from β-oxidation of fatty acids in intact metabolic systems[9–12]. However, Krebs[13] has suggested that acetyl-CoA derived from fatty acid oxidation can be directed toward ketogenesis by the inhibition of citrate synthase by ATP generated during the oxidation of various substrates in rat liver homogenates.

The experiments described in this communication were performed to assess the effect of α-ketoglutarate, one of the substrates used in Krebs' liver homogenate experiments, on the rate of oxidation of L(—)-palmitylcarnitine in isolated rat liver mitochondria.

MATERIALS AND METHODS

Rat liver mitochondria were isolated in 0.225 M mannitol, 0.075 M sucrose and 0.1 mM ethylene glycolbis(aminoethyl) tetraacetate (EGTA) as described by Chance and Mela[14]. Oxygen consumption was measured in a 2.0-ml lucite reaction chamber using a Clark-type oxygen electrode (Yellow Springs No. 5331). Absorption measurements of the intramitochondrial reduced pyridine nucleotides were accomplished using a Perkin–Elmer Model 356 dual beam/split beam spectrophotometer using the wave-length pair 340–374 nm. Samples of the mitochondrial reaction mixtures (1.0) were withdrawn from the oxygen electrode chamber (volume 6.0 ml) with an Eppendorf automatic pipet and the protein was precipitated with $HClO_4$ (final concentration 6%, w/v). The samples were neutralized with 3 M K_2CO_3 *plus* 0.5 M triethanolamine prior to the measurement of various intermediates. Metabolic intermediates were measured using the enzymatic–fluorometric procedures described by Williamson and Corkey[15]. Enzymes used in these assays were obtained from Boehringer Mannheim Corporation. Mitochondrial protein concentrations were estimated using a biuret procedure[16]. L(—)-Palmitylcarnitine was prepared using a modification of the procedure described by Bremer[17]. L(—)-Carnitine was obtained from the Otsuka Pharmaceutical in Osaka, Japan. The *p*-trifluoromethoxyphenylhydrazone of carbonyl cyanide (FCCP) was the generous gift of Dr P. G. Heytler of the E. I. DuPont DeNemours Company. Bovine serum albumin was obtained from Sigma Chemical Company and was defatted according to the procedure of Chen[18]. Atractyloside was purchased from Calbiochem. Other chemicals were of reagent grade and were purchased from Sigma Chemical Company.

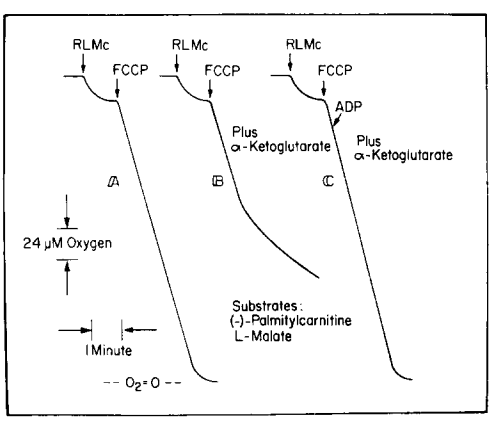
RESULTS

Both coupled and uncoupled rat liver mitochondria oxidizing L(—)-palmitylcarnitine and L-malate in the presence of monofluorocitrate to block the aconitase reaction of the citric acid cycle synthesize citrate at a rapid rate[8]. Approx. 90% of the oxygen consumption under these conditions can be attributed to citrate formation; the remainder of the oxygen consumption is due to the formation of aceto-

acetate while only negligible amounts of β-hydroxybutyrate are formed under these conditions. Fig. 1, Curve A, illustrates the oxygen consumption trace of a suspension of uncoupled rat liver mitochondria oxidizing 50 μM L(—)-palmitylcarnitine *plus* 1 mM L-malate. The respiration rate (49.1 nmoles O_2 per min per mg protein) was linear following the addition of the uncoupler FCCP (1.0 μM). The rate of oxygen consumption (not shown in Fig. 1) using 1.0 mM ADP instead of FCCP to stimulate respiration in this mitochondrial system was also linear and was 47.4 nmoles O_2 per min per mg protein. Fig. 1, Curve B, demonstrates the effect of adding 1.0 mM α-ketoglutarate on the respiration rate using the substrate couple L(—)-palmitylcarnitine *plus* L-malate. Upon the addition of uncoupler the rate of oxygen consumption in the presence of α-ketoglutarate was linear for approx. 1 min followed by a gradual and pronounced inhibition of the oxidation rate.

Fig. 1, Curve C, indicates that the respiratory inhibition caused by the addition of α-ketoglutarate can be prevented by the addition of 1.0 mM ADP to the reaction mixture. ADP addition to the uncoupled mitochondria oxidizing L(—)-palmitylcarnitine *plus* L-malate restored the rapid, linear rate of oxygen consumption (63.6 nmoles O_2 per min per mg protein). If ADP was added following the establishment of the inhibited state, the inhibition of oxygen consumption was only partially released.

Fig. 2 demonstrates that the inhibition of L(—)-palmitylcarnitine *plus* L-malate oxidation was dependent on the concentration of α-ketoglutarate added to the reaction mixture. Concentrations of α-ketoglutarate exceeding 1.0 mM did not provide a greater inhibition of the respiration rate. Variation of the concentration



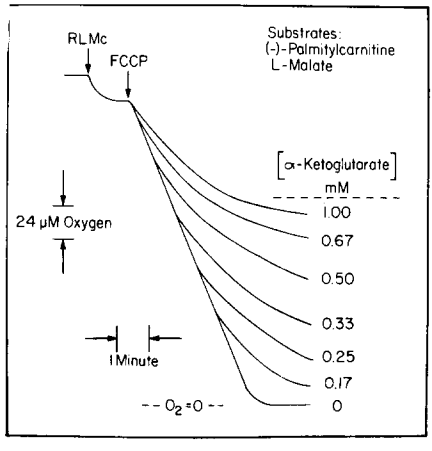


Fig. 1. The effect of α-ketoglutarate addition on L(—)-palmitylcarnitine *plus* L-malate oxidation in uncoupled rat liver mitochondria (RLMc). The reaction mixture contained 100 mM KCl, 50 mM sucrose, 20 mM Tris–chloride and 5 mM Tris–phosphate. The mitochondrial concentration was 1.24 mg protein/ml. Other additions to the incubations: 4 mg/ml defatted bovine serum albumin, 0.25 μM FCCP, 1.0 mM α-ketoglutarate, 500 mM ADP, 50 μM L(—)-palmitylcarnitine and 1.25 mM L-malate. Oxygen consumption was measured using a Clark-Type oxygen electrode in a lucite reaction chamber, volume 2.0 ml.

Fig. 2. The effect of α-ketoglutarate concentration on L(—)-palmitylcarnitine *plus* L-malate oxidation in uncoupled rat liver mitochondria (RLMc). Reaction conditions were the same as described in the legend for Fig. 1. The concentration of α-ketoglutarate was increased from 0 to 1.0 mM.

of L-malate over the range 0.5–7.5 mM had no effect on the inhibition of oxygen consumption by α-ketoglutarate.

Fig. 3 illustrates that the rate of citrate formation was markedly inhibited concomitant with the inhibition of oxygen consumption when α-ketoglutarate was included in the reaction mixture. Rapid, linear rates of citrate formation were observed in the uncoupled mitochondria using L(—)-palmitylcarnitine and L-malate as substrates (see Trace A, control). The apparent rates of citrate synthesis observed in these experiments which were performed in the absence of a monofluorocitrate block were lower by a factor of two than those reported previously[8]. This merely indicates that some citrate was oxidized in the citric acid cycle and did not accumulate as rapidly in the absence of monofluorocitrate.

The addition of α-ketoglutarate caused a gradual and nearly complete inhibition of citrate formation. The time course of the inhibition of citrate formation paralelled the inhibition of oxygen consumption.

Inclusion of 1.0 mM ADP in the reaction mixture not only prevented the establishment of the α-ketoglutarate mediated inhibition of citrate production but caused an increase in the rate of citrate production from the control value of 9.0 to 15.3 nmoles citrate per min per mg protein. Under the conditions of these incubations, *i.e.* uncoupled mitochondrial oxidizing L(—)-palmitylcarnitine *plus* L-malate, very small amounts of either acetoacetate or β-hydroxybutyrate were formed (less than 1.0 nmole per min per mg protein). Upon initiation of the respiratory inhibition by α-ketoglutarate there was no detectable increase in the rate of acetoacetate pro-

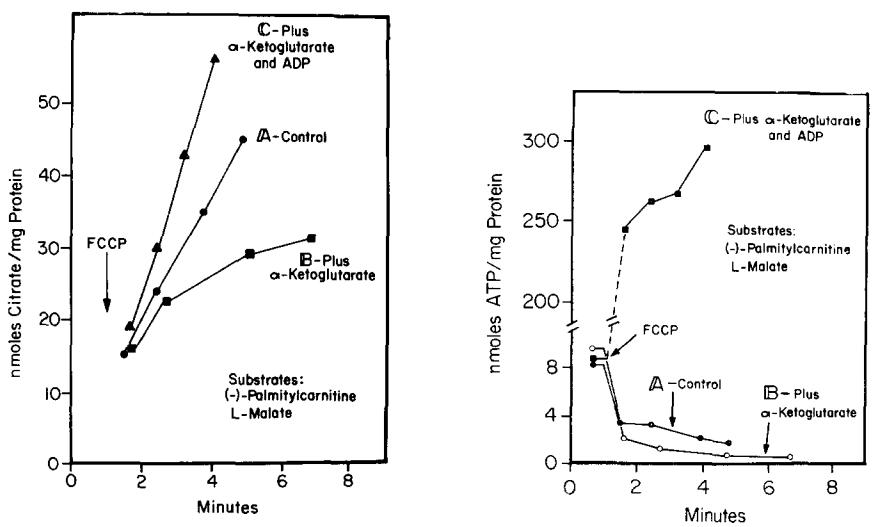


Fig. 3. The effect of α-ketoglutarate addition on the rate of citrate formation. Reaction conditions were the same as described in Fig. 1 with the exception that the reaction volume was 6.0 ml. Samples (1.0 ml) were removed from the reaction chamber, the protein was precipitated with 6% (v/w) $HClO_4$ and assays were performed as described in the methods on the neutralized extracts.

Fig. 4. The effect of α-ketoglutarate induced inhibition of L(—)-palmitylcarnitine *plus* L-malate oxidation on the ATP level of the uncoupled rat liver mitochondria. Reaction conditions were the same as described in Figs 1 and 3.

duction suggesting that the addition of α-ketoglutarate did not cause a switch from the oxidation of acetyl units in the citric acid cycle to the conversion of these two-carbon units to ketone bodies.

Addition of uncoupler to the mitochondria in the control experiment (see Trace A, Fig. 4) caused a rapid decrease in the intramitochondrial ATP concentration within the first minute after FCCP addition. The ATP level in the reaction inhibited by α-ketoglutarate showed a similar decrease after FCCP addition and maintained a slightly lower level of ATP than the control experiment during the remainder of the incubation period. In the experiment in which the inhibition of the respiratory rate and citrate production was prevented by the addition of ADP, the ATP level was 100-fold higher than either the control or the α-ketoglutarate inhibited experiments.

The high rate of citrate formation (15.3 nmoles per min per mg protein) in the experiment to which ADP was added indicates that the rate of citrate formation did not appear to be inhibited by the elevated level of ATP compared to the control which had low ATP. This observation would seem to contradict the results of Garland *et al.*[4,5] and confirm those of Olson and Williamson[8] which suggest that citrate synthase in isolated liver mitochondria is insensitive to the ATP level.

The addition of the uncoupler, FCCP, caused an immediate and rapid oxidation of reduced pyridine nucleotides as evidenced by the decrease in absorbance of the mitochondrial suspension at 340 nm shown in Curve A of Fig. 5. The redox state remained oxidized in this uncoupled state until anaerobiosis occurred in the mitochondrial suspension at which time the absorbance at 340 nm increased indicating the reduction of NADH and NADPH. Curve B of Fig. 5 demonstrates that the initiation of the inhibited state by α-ketoglutarate addition caused a rapid but transient increase in absorption at 340 nm followed by a slower decrease in the absorbance attaining a lower level than was observed in Curve A as the inhibition of respiration and citrate production occurred. The significance of the more oxidized redox state during the inhibited state will be discussed below.

Addition of ADP to the uncoupled mitochondria oxidizing L(—)-palmitylcarnitine *plus* L-malate markedly changed the response of the mitochondrial sus-

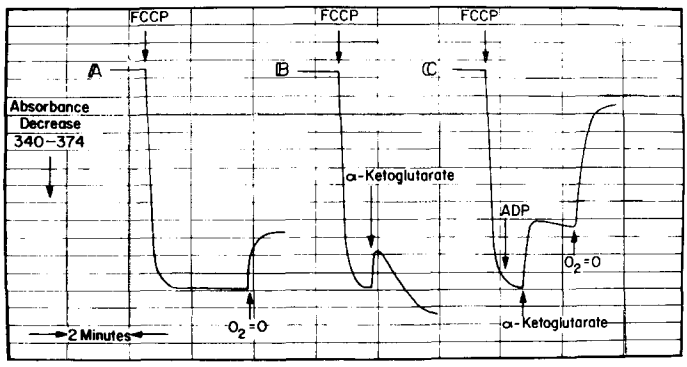


Fig. 5. The effect of α-ketoglutarate on the redox state of the intramitochondrial NAD of uncoupled rat liver mitochondria oxidizing L(—)-palmitylcarnitine *plus* L-malate. Reaction conditions were the same as described in Fig. 1. Absorption measurements were made as described in the methods using the wavelength pair 340–374 nm.

pension to α-ketoglutarate addition. α-Ketoglutarate addition following the addition of ADP caused a rapid and significant reduction of the pyridine nucleotides (Curve C, Fig. 5). In contrast to the α-ketoglutarate inhibited mitochondria (Curve B, Fig. 5), the redox state attained following α-ketoglutarate addition was maintained until the mitochondrial suspension became anaerobic whereupon the absorbance of the suspension at 340 nm increased to the level observed prior to the addition of the uncoupler, FCCP.

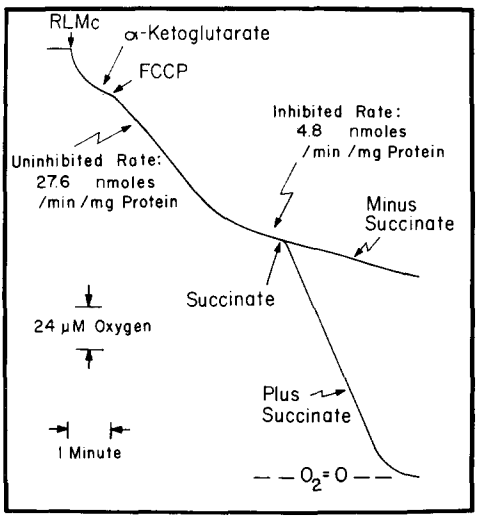


Fig. 6. The effect of succinate addition to rat liver mitochondria (RLMc) in the α-ketoglutarate induced inhibitory state. Reaction conditions were the same as described in Fig. 1 with the exception that L(−)-palmitylcarnitine and L-malate were omitted and the α-ketoglutarate concentration was 1.2 mM. 2.5 mM succinate was added after the α-ketoglutarate oxidation was inhibited. The effects of adding substrates other than succinate to the incubation mixture in the inhibited state are listed in Table I. The mitochondrial concentration was 1.05 mg protein/ml.

At this point it is crucial to consider the specificity of the inhibitory effect of α-ketoglutarate addition on the oxidation of L(−)-palmitylcarnitine *plus* L-malate. A series of experiments were performed in which rat liver mitochondria were incubated with only the substrate–inhibitor, α-ketoglutarate, and respiration was stimulated with FCCP (Fig. 6). Following uncoupler addition, α-ketoglutarate was oxidized at a linear rate for approx. 2 min. This "uninhibited rate" was 27.6 nmoles per min per mg protein. The uninhibited rate of α-ketoglutarate oxidation was followed by a spontaneous, abrupt, 5-fold inhibition of the oxidation rate. This inhibited rate was 4.8 nmoles per min per mg protein. The inhibition of α-ketoglutarate oxidation seen in Fig. 6 was characteristic of that seen in Figs 1 and 2 for the α-ketoglutarate induced inhibition of L(−)-palmitylcarnitine *plus* L-malate oxidation. After the inhibited rate had been attained, various substrates or combinations of substrates were added. The addition of succinate in this type of experiment is illustrated in Fig. 6. Succinate addition to the inhibited rate markedly stimulated the rate of oxygen consumption presumably due to the oxidation of the added succinate. The inclusion of malonate in the incubation medium prevented the stimulation of respiration by succinate. Table I illustrates the rates of oxidation of various substrates in

TABLE I

THE EFFECT OF α-KETOGLUTARATE ON THE OXIDATION OF VARIOUS SUBSTRATES IN ISOLATED RAT LIVER MITOCHONDRIA

Uncoupled mitochondria oxidizing various substrates were incubated in the presence and absence of α-ketoglutarate in experiments as illustrated and described in Fig. 6. Respiration rates were measured using an oxygen electrode as described in the Methods.

Substrate	*Concn (mM)*	*Respiration rate (nmoles O_2 per min per mg protein)*
1. α-Ketoglutarate	1.2	27.6 (uninhibited rate) 4.8 (inhibited rate)
2. Pyruvate *plus*	1.2	9.6
L-malate	1.2	
+ α-ketoglutarate	1.2	4.8
3. L(−)-Palmitylcarnitine *plus*	0.06	51.6
L-malate	1.2	
+ α-ketoglutarate	1.2	10.8
4. Octanoate *plus*	0.12	32.4
L-malate	1.2	
+ α-ketoglutarate	1.2	7.2
5. Glutamate *plus*	2.5	24.0
L-malate	2.5	
+ α-ketoglutarate	1.2	4.8
6. β-Hydroxybutyrate	2.5	14.0
+ α-ketoglutarate	1.2	6.0
7. Isocitrate	2.5	21.6
+ α-ketoglutarate	1.2	6.0
8. Succinate	2.5	50.4
+ α-ketoglutarate	1.2	66.0

the presence as well as the absence of the α-ketoglutarate induced inhibitory state. It can be readily observed that the oxidation of all NAD-linked substrates tested in this experiment was significantly inhibited during the α-ketoglutarate induced inhibitory state. Succinate, a flavin-linked substrate was the only substrate whose oxidation was not affected by the α-ketoglutarate inhibition.

The data presented in Fig. 1 indicated that ADP addition prevented the establishment of the α-ketoglutarate inhibition of L(−)-palmitylcarnitine *plus* L-malate oxidation. Fig. 7 demonstrates that ADP also prevented the respiratory inhibition occurring during α-ketoglutarate oxidation in uncoupled mitochondria (compare Curves A and B of Fig. 7). Addition of atractyloside to the mitochondrial experiment (Curve C) appeared to intensify the inhibition of respiration in the control experiment. As would be expected if atractyloside inhibitis the exchange or transport of adenine nucleotides across the mitochondrial membranes, the inclusion of atractyloside and ADP in the incubation mixture prevented the effect of ADP on the α-ketoglutarate induced inhibition of respiration. In short, the mechanism by which ADP affects this system is within the atractyloside sensitive barrier. It was also observed (unpublished observation) that the addition of oligomycin to uncoupled mitochondria oxidizing either α-ketoglutarate or α-ketoglutarate *plus* L(−)-palmitylcarnitine and L-malate accentuated the inhibitory state induced during the oxidation of the α-ketoglutarate.

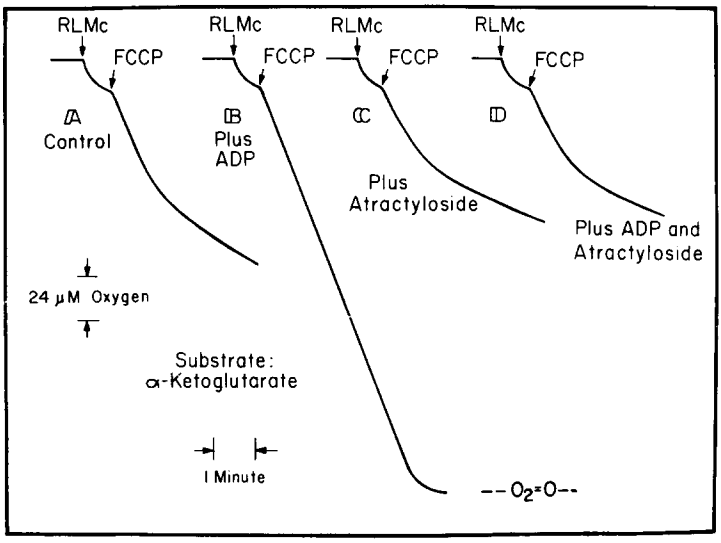


Fig. 7. The effect of atractyloside on the ADP release of α-ketoglutarate induced respiratory inhibition. Reaction conditions were the same as described in Fig. 6 with the exception that 1.0 mM ADP and atractyloside (10 μg/ml) were included in the incubations where indicated. The mitochondrial concentration was 1.44 mg protein/ml. Rat liver mitochondria = RLMc.

DISCUSSION

The cell must strike an intricate balance between energy generation and energy utilization or dissipation in order to maintain energetic homeostasis. A cellular energetic balance is possible only as a result of the evolution of various regulatory properties of the component enzymes and metabolic pathways of the cell. The significance of the regulation of the oxidative reactions of the citric acid cycle derives from the fact that much of the energy generating capacity of the cell is obligatorily coupled to this cyclic series of reactions. Two general modes of regulation of the citric acid cycle have been described: (a) a respiratory or energy coupling control, and (b) direct and specific interactions of effector molecules (both activators and inhibitors) with various cycle enzymes. The respiratory or energy coupling control of the citric acid cycle is expressed by either a restriction or a facilitation of the four dehydrogenase reactions of the cycle as a result of the relative availability of the oxidized forms of the nicotinamide and flavin coenzymes (NAD^+ and FAD) involved in the dehydrogenase reactions of the cycle. The availability of oxidized NAD^+ and FAD may depend upon the state of reduction of the electron transfer carriers, or alternatively upon the phosphorylation state ($[ATP]/[ADP][P_i]$) of the adenine nucleotides of the mitochondrial compartment because of the coupling relationship which exists between the processes of electron transport and the oxidative phosphorylation of ADP. Respiratory control would also be exerted upon the processes of β-oxidation of fatty acids and the oxidation of pyruvate to acetyl-CoA because of the dependence of these reactions on the supply of NAD^+.

Various citric acid cycle enzymes including citrate synthase[3] and isocitrate dehydrogenase[19–21] have been shown to be directly and specifically activated and/or

inhibited by various effector molecules. These specific effector–enzyme relationships have been established on the basis of *in vitro* experiments using purified enzymes from a variety of sources. It is essential, however, to collect data in more complex biological systems to either confirm or refute the isolated enzyme data.

When considering the regulation of the fate of acetyl units in a tissue such as liver, *i.e.* oxidation in the citric acid cycle or formation of ketone bodies; a crucial distinction must be made between regulatory effects which are specific for the citric acid cycle and those which affect the general respiratory process of the mitochondrion. Regulatory effects involving respiratory chain would influence not only the generation of acetyl-CoA, *e.g.* fatty acid or pyruvate oxidation; but also the utilization of acetyl-CoA in the citric acid cycle. In short, a general effect on the respiratory system would not be expected to cause a switch or change in the fate of acetyl units while a specific inhibition of one of the reactions of the citric acid cycle would be expected to divert acetyl units to ketogenesis.

The experiments described in this communication were prompted by the interesting observation of Krebs[13] that α-ketoglutarate exerted a ketogenic effect when added to a fatty acid oxidating rat liver homogenate. Krebs asserted that ATP generated from the oxidation of α-ketoglutarate or other substrates inhibited the mitochondrial citrate synthase, thus diverting acetyl units from the citric acid cycle toward ketone body formation. This observation coupled with the experiments of Olson and Williamson[8] demonstrating that the rate of citrate synthesis in isolated rat liver mitochondria was insensitive to the intramitochondrial ATP level suggested that the ATP generated *via* α-ketoglutarate oxidation was "different" in its effect on citrate synthase. The idea that GTP formed during substrate level phosphorylation following α-ketoglutarate oxidation and/or a pool of ATP in direct communication with this GTP may exert an inhibitory effect on citrate synthase, which is not observed for ATP generated *via* oxidative phosphorylation, seemed worthy of experimental consideration.

The oxygen electrode traces shown in Figs 1 and 2 demonstrate that the oxidation of L(—)-palmitylcarnitine *plus* L-malate was markedly inhibited by the addition of α-ketoglutarate to uncoupled rat liver mitochondria. This inhibition appeared only after a definite lag phase, was dependent upon the concentration of α-ketoglutarate, and could be prevented by the inclusion of ADP in the incubation mixture. The fact that α-ketoglutarate oxidation inhibited both the formation of citrate (Fig. 3) and did not result in an enhanced rate of ketone body formation indicated that α-ketoglutarate did not cause a switch in the fate of acetyl units from citrate formation to ketogenesis.

As cautioned earlier in this discussion, the α-ketoglutarate induced inhibition of fatty acid oxidation seemed to be a general effect on mitochondrial respiration rather than a specific inhibition of an enzyme such as citrate synthase.

Addition of α-ketoglutarate and the induction of the inhibitory state did not cause an increase in the intramitochondrial ATP level (Fig. 4, Curve B) which would be necessary if an ATP inhibition of citrate synthase was occurring. The fact that the maximal rate of citrate synthesis (Fig.3, Curve C) occurred in the presence of ADP and α-ketoglutarate, a metabolic situation characterized by a high ATP level in the incubation mixture, indicates the ATP level can not be correlated with the rate of citrate synthesis.

The inhibitory state induced by the oxidation of α-ketoglutarate may also be characterized by a progessive oxidation of the intramitochondrial NAD^+. This oxidized redox state was prevented by inclusion of ADP in the incubation medium. The absorbance measurements shown in Fig. 5 indicate that reducing equivalents from either the oxidation of α-ketoglutarate or the L(—)-palmitylcarnitine *plus* L-malate were not available to the respiratory chain in the inhibited state. This observation implies that the site of inhibition was prior to the NAD level of the respiratory chain.

That the inhibitory state induced by α-ketoglutarate oxidation was a general effect on the oxidation of NAD-linked substrates is indicated by the experiments described in Fig. 6 and Table I. The oxidation of all NAD-linked substrates or substrate couples tested in this experiment was inhibited in the α-ketoglutarate-induced inhibitory state. Succinate oxidation, on the other hand, was not affected by α-ketoglutarate.

The ability of ADP to prevent completely the induced inhibitory state, while ATP, AMP, and other exogenous nucleoside diphosphates were without effect, suggested the possibility that the inhibitory effect may result from increased intramitochondrial levels of GTP formed during the metabolism of α-ketoglutarate. Exogenous ADP would react with this GTP in the nucleoside diphosphokinase reaction and would result in a lowering of GTP and a prevention of the inhibitory effect:

$$ADP + GTP \rightleftharpoons GDP + ATP$$

This would imply that the GTP formed from α-ketoglutarate oxidation was not readily accessible to the uncoupling action of FCCP. The observation that atractyloside accentuated the inhibitory state and completely prevented the effect of ADP on the inhibitory state is consistent with the reported effects of atractyloside on both the transfer or exchange of adenine nucleotides across the mitochondrial membrane[22] and the inhibition of the nucleoside diphosphokinase[23]. Also consistent with the postulated GTP mechanism is the fact that oligomycin accentuated the inhibitory effect of α-ketoglutarate and did not affect the ADP prevention of the inhibition. In this regard Davis[24] reported an inhibition of α-ketoglutarate oxidation in guinea pig heart mitochondria by oligomycin. This inhibition was released by both Mg^{2+} and acetoacetate but not by ADP added after the addition of oligomycin. Because of the differences in the experimental system, *i.e.* type of mitochondria and the order of addition and concentrations of the various components, it is difficult to compare these experiments to those of Davis.

Experiments are currently being performed to substantiate that increased intramitochondrial GTP levels are inhibitory to NAD-linked substrate oxidation in isolated mitochondria. It is essential to eliminate GDP deprivation of the succinate thiokinase step and/or feedback inhibition by succinyl-CoA[25] of the α-ketoglutarate dehydrogenase as possible causes of the inhibition of NAD-linked substrate oxidation. At this point, however, it is difficult to visualize how either of these possibilities may affect the oxidation of substrates other than α-ketoglutarate.

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REFERENCES

1 J. A. Hathaway and D. E. Atkinson, *Biochem. Biophys. Res. Commun.*, 20 (1965) 661.
2 D. Shepherd and P. B. Garland, *Biochem. J.*, 114 (1969) 597.
3 N. O. Jangaard, J. Unkeless and D. E. Atkinson, *Biochim. Biophys. Acta*, 151 (1968) 225.
4 P. B. Garland, in T. W. Goodwin, *Metabolic Roles of Citrate*, Academic Press, New York, 1968, p. 41.
5 P. B. Garland, D. Shepherd and D. G. Nicholls, in E. Quagliariello, S. Papa, E. C. Slater and J. M. Tager, *Mitochondrial Structure and Compartmentation*, Adriatica Editrice, Bari, 1967, p. 424.
6 O. Shepherd, D. W. Yates and P. B. Garland, *Biochem. J.*, 97 (1965) 38C.
7 J. R. Williamson, M. S. Olson, E. T. Browning and R. Scholz, in E. Quagliariello, E. C. Slater, S. Papa and J. M. Tager, *Energy Level and Metabolic Control in Mitochondria*, Adriatica Editrice, Bari, 1969, p. 207.
8 M. S. Olson and J. R. Williamson, *J. Biol. Chem.*, 246 (1971) 7794.
9 O. Wieland, F. Matschinsky, G. Löffler and U. Müller, *Biochim. Biophys. Acta*, 53 (1961) 412.
10 J. R. Williamson, M. S. Olson, B. E. Herczeg and H. S. Coles, *Biochem. Biophys. Res. Commun.*, 27 (1967) 595.
11 A. Van Tol, *Biochim. Biophys. Acta*, 223 (1970) 429.
12 S. G. Van den Bergh, in *Abstracts of the Colloquium on Bioenergetics: Energy Transduction in Respiration and Photosynthesis*, Adriatica Editrice, Bari, 1971, p. 19.
13 H. A. Krebs, in G. Weber, *Advances in Enzyme Regulation*, Vol. 8, Pergamon Press, New York, 1970, p. 335.
14 B. Chance and L. Mela, *J. Biol. Chem.*, 241 (1966) 4588.
15 J. R. Williamson and B. E. Corkey, in J. M. Lowenstein, *Methods in Enzymology*, Vol. 13, Academic Press, New York, 1968, p. 434.
16 E. Layne, in S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, Vol. 3, Academic Press, New York, 1957, p. 447.
17 J. Bremer, *J. Biol. Chem*, 237 (1962) 3628.
18 R. F. Chen, *J. Biol. Chem.*, 242 (1967) 173.
19 H. Goebell and M. Klingenberg, *Biochem. Z.*, 340 (1964) 441–464.
20 J. A. Hathaway and D. E. Atkinson, *J. Biol. Chem.*, 238 (1963) 2875.
21 R. I. Chen and G. W. E. Plaut, *Biochemistry*, 2 (1963) 1023.
22 A. Bruni, S. Luciani and C. Bortignon, *Biochim. Biophys. Acta*, 97 (1965) 434.
23 D. W. Allmann and R. A. Harris, *Fed. Proc.*, 26 (1967) 456.
24 J. E. Davis, *Biochim. Biophys. Acta*, 96 (1965) 528.
25 P. B. Garland, *Biochem. J.*, 92 (1964) 10C.