Stimulation of the Glycine Cleavage System by Short-Chain Fatty Acids in Isolated Rat Liver Mitochondria†

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ABSTRACT: The effect of short-chain fatty acids on glycine catabolism by the glycine cleavage system was investigated in isolated rat liver mitochondria. Metabolic flux through the glycine cleavage system was monitored by measuring the production of $^{14}CO_2$ from [1-^{14}C]glycine. Propionate, butyrate, pentanoate, and octanoate, and to a lesser extent acetate, all significantly stimulated $^{14}CO_2$ production from [1-^{14}C]glycine by isolated rat liver mitochondria maintained in state 4. Concomitant with the stimulation of $^{14}CO_2$ production was a decrease in the measured intramitochondrial content of NADPH which we have previously demonstrated correlates with the metabolic flux through the glycine cleavage system [Hampson, R. K., Barron, L. L., & Olson, M. S. (1983) *J. Biol. Chem. 258*, 2993-2999]. The propionate-mediated stimulation of mitochondrial $^{14}CO_2$ production from [1-^{14}C]glycine was not diminished by the addition of L-carnitine but was abrogated nearly completely by the addition of oligomycin. Incubation of the mitochondria with short-chain fatty acids evoked a large decrease in the measured intramitochondrial ATP content and a large increase in the AMP content. However, manipulation of the intramitochondrial adenine nucleotide profile demonstrated that no direct correlation existed between ATP, ADP, or AMP and $^{14}CO_2$ production from [1-^{14}C]glycine. These experimental results indicate that short-chain fatty acid oxidation causes the oxidation of the NADP(H) redox couple in isolated rat liver mitochondria, resulting in the stimulation of the metabolic flux through the glycine cleavage system. This stimulation of glycine cleavage was initiated by a rapid depletion of intramitochondrial ATP during activation of the fatty acids to their respective acyl coenzyme A derivatives, decreasing the mitochondrial phosphate potential and partially uncoupling the mitochondria. In the absence of a strongly reducing substrate, this sequence of events results in the flow of electrons from NADPH to NAD^+ via the energy-linked transhydrogenase.

The glycine cleavage system catalyzes the tetrahydrofolate-dependent cleavage of glycine to yield carbon dioxide, ammonia, N^5,N^{10}-methylenetetrahydrofolate, and 1 reducing equivalent as NADH + H^+ and represents the primary pathway by which glycine is catabolized in mammals (Yoshida & Kikuchi, 1972). This system, located exclusively in the mitochondrial compartment (Motokawa & Kikuchi, 1971), is comprised of the following four distinct protein components (Kikuchi, 1973): a pyridoxal phosphate containing glycine decarboxylase, a lipoic acid containing aminomethyl transferase, an N^5,N^{10}-methylenetetrahydrofolate-synthesizing enzyme, and the flavoprotein dihydrolipoyl dehydrogenase, likely existing as a multienzyme complex (Hiraga et al., 1972) loosely associated with the inner mitochondrial membrane (Motokawa & Kikuchi, 1971). The composition and reaction mechanism of the glycine cleavage system bear a marked similarity to those of the various α-keto acid dehydrogenase complexes (Olson et al., 1981).

Regulatory mechanisms governing the rate of hepatic glycine catabolism are not well understood. We have reported that metabolic flux through the glycine cleavage system in isolated fully functional rat liver mitochondria is very sensitive to the oxidation-reduction state of the mitochondrial NAD(H) and NADP(H) redox couples (Hampson et al., 1983). Conditions which lead to an oxidation of the mitochondrial respiratory chain (e.g., state 3, uncouplers, or propionate) stimulate mitochondrial glycine decarboxylation, while reducing conditions (e.g., respiratory inhibitors or strongly reducing substrates) inhibit this process. Independent manipulation of the NAD(H) and NADP(H) redox couples demonstrated the sensitivity of flux through the glycine cleavage system to each redox couple.

The principal evidence that metabolic flux through the glycine cleavage system, in isolated rat liver mitochondria, is sensitive to the oxidation-reduction state of the NADP(H) redox couple was the observation that the three-carbon fatty acid propionate stimulated mitochondrial glycine decarboxylation with a concomitant decrease in the measured intramitochondrial content of NADPH but little or no change in the measured NADH content. The present study was performed in an attempt to elucidate the mechanism by which propionate oxidizes the NADP(H) redox couple in isolated rat liver mitochondria with subsequent stimulation of the metabolic flux through the glycine cleavage system.

Materials and Methods

Mitochondria employed in these studies were isolated by the method of Schneider & Hogeboom (1950) from livers obtained from male Sprague-Dawley rats (200-250 g) fed a standard laboratory chow and water ad libitum. The liver was homogenized in a medium consisting of mannitol (0.225 M), sucrose (0.075 M), and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA)[1] (0.1 mM). Subsequent washings employed a similar medium without EGTA and contained HEPES (5 mM), pH 7.4. The mitochondrial protein concentration was estimated by using a biuret procedure (Gornall et al., 1948) with bovine serum albumin as a standard.

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[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone; HPLC, high-performance liquid chromatography; CoA, coenzyme A.

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Metabolic flux through the glycine cleavage system was monitored by measuring the release of $^{14}CO_2$ from [1-^{14}C]-glycine. Previously we demonstrated that the glycine cleavage system is responsible for greater than 95% of the observed $^{14}CO_2$ production from [1-^{14}C]glycine (Hampson et al., 1983). Mitochondrial incubations were performed, and the production of $^{14}CO_2$ from [1-^{14}C]glycine was measured as described previously. The incubation medium, saturated with 100% oxygen prior to addition to mitochondria, consisted of potassium chloride (100 mM), mannitol (50 mM), sucrose (20 mM), glucose (30 mM), potassium phosphate (10 mM), magnesium chloride (1 mM), EGTA (0.1 mM), pyridoxal phosphate (0.175 mM), HEPES (25 mM), pH 7.4, and from 1.2 to 1.5 mg of mitochondrial protein/mL.

The reduced pyridine nucleotides NADH and NADPH were measured as described previously using an enzymatic–fluorometric method (Williamson & Corkey, 1969). The intramitochondrial adenine nucleotides were measured as follows. Samples (0.8 mL) of three identical mitochondrial incubations (incubation time = 7.5 min) were layered on top of silicone oil (0.5 M Versilube[2] F-50, specific gravity = 1.05) which was layered upon 0.1 mL of perchloric acid (10.5%) containing ethanol (10%) in Eppendorf-type centrifuge tubes (1.5 mL). Following centrifugation for 2 min in an Eppendorf table-top centrifuge, the layers of incubation medium and silicone oil were removed by aspiration, and the perchloric acid layers were removed as completely as possible, combined into one centrifuge tube, and vortexed. Following centrifugation for 2 min, 0.2 mL of the extract (approximately 0.3 mL) was carefully removed, avoiding any silicone oil or protein pellet, transferred to another tube, and neutralized (pH 6.0–7.0) with K_2CO_3 (3 M) containing K_2HPO_4 (0.25 M). The neutralized extracts were centrifuged for 2 min, and 50-μL samples were analyzed by HPLC using a slight modification of the procedure of Garrison et al. (1982) on a Whatman Partasil 10 SAX anion-exchange column. The concentrations of the nucleotide standards were assayed enzymatically (Williamson & Corkey, 1969). Coenzyme A and its derivatives were measured in a very similar manner except the perchloric acid layer contained 2 mM dithiothreitol (added immediately prior to use) instead of ethanol. Following extraction and neutralization (pH 4.0–6.0), 100-μL samples were analyzed by HPLC employing the method of DeBuysere & Olson (1983) on a Custom LC ODS II reverse-phase column. HPLC-grade solvents and buffers were used in all HPLC analyses.

Radiolabeled [1-^{14}C]glycine was purchased from ICN Chemical and Radioisotope Division. Phenylethylamine and Liquiscint aqueous counting scintillation fluid were purchased from National Diagnostics. All nucleotides were obtained from P-L Biochemicals, and oligomycin was obtained from Sigma. FCCP was obtained from Pierce and carnitine from Otsuka Pharmaceutical Factory (Osaka, Japan).

Results and Discussion

In a previous publication, we reported that the three-carbon fatty acid propionate stimulated the production of $^{14}CO_2$ from [1-^{14}C]glycine, while there occurred a concomitant decrease in the measured intramitochondrial NADPH content (Hampson et al., 1983). On the basis of this observation, we concluded that metabolic flux through the mitochondrial glycine cleavage system, which we demonstrated to be responsible for nearly all the $^{14}CO_2$ production from [1-^{14}C]-glycine, was sensitive to the oxidation–reduction state of the mitochondrial NADP(H) redox couple.

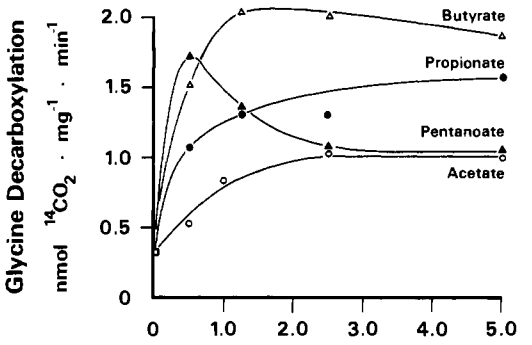

FIGURE 1: Effect of acetate (○), propionate (●), butyrate (△), and pentanoate (▲) on $^{14}CO_2$ production from [1-^{14}C]glycine by rat liver mitochondria. The mitochondria were incubated as described under Materials and Methods under state 4 conditions. The glycine concentration was 5 mM.

Table I: Effect of Acetate, Propionate, Butyrate, and Pentanoate on the Intramitochondrial Content of NADH and NADPH in Rat Liver Mitochondria[a]

incubation conditions	content (nmol·mg of mitochondrial protein^{-1}) NADH	NADPH
no addition	0.28 ± 0.02	4.08 ± 0.36
acetate, 2.5 mM	0.18 ± 0.03	2.56 ± 0.13
propionate, 2.5 mM	0.04 ± 0.02	1.35 ± 0.12
butyrate, 2.5 mM	0.09 ± 0.06	1.25 ± 0.13
pentanoate, 2.5 mM	0.25 ± 0.13	2.15 ± 0.19

[a] The mitochondrial NADH and NADPH were extracted and quantitated as described under Materials and Methods. The mitochondria were maintained in state 4 with 5 mM glycine. The values are presented as means ± the SD for three independent determinations.

The experiment depicted in Figure 1 was performed in order to assess whether the stimulation of mitochondrial glycine decarboxylation by propionate is specific for propionate. Production of $^{14}CO_2$ from [1-^{14}C]glycine by rat liver mitochondria, maintained in state 4, was measured in the absence and presence of acetate, propionate, butyrate, and pentanoate. Presented in Table I are the measured intramitochondrial levels of NADH and NADPH in the absence and presence of these fatty acids at 2.5 mM. Each fatty acid stimulated glycine decarboxylation by rat liver mitochondria and caused a substantial decrease in the intramitochondrial content of NADPH which seemed to be proportional to the extent of stimulation of glycine decarboxylation. Only a small absolute change in the NADH content was observed upon incubation with the various fatty acids compared to the maximal possible change of the NAD(H) redox state under highly reduced conditions. For comparison to a highly reduced state, the intramitochondrial contents of NADH and NADPH in the presence of α-ketoglutarate, 5 mM, under state 4 conditions are 2.82 ± 0.34 and 5.59 ± 0.49 nmol·mg of mitochondrial protein^{-1}, respectively. The observation that freshly isolated rat liver mitochondria exhibit a high level of intramitochondrial NADPH and a relatively low level of NADH has been reported by others (Klingenberg & Slencska, 1959) and likely is the result of the nature of the energy-linked transhydrogenase [for a review, see Rydstrom (1977)].

The decrease in mitochondrial glycine decarboxylation at higher concentrations of the longer chain fatty acids (i.e., butyrate and pentanoate) may be the result of a number of factors. First of all, at higher concentrations, these fatty acids no longer exhibit a simple, monodisperse solution structure but are characterized by a more complicated structure. Second,

[2] Versilube F-50 silicone oil was the generous gift of the General Electric Corp., Silicone Products Division, Waterford, NY.

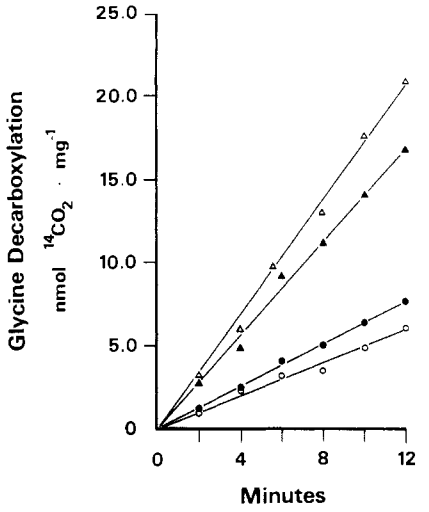

FIGURE 2: Effect of carnitine on the stimulation, by propionate, of $^{14}CO_2$ production from [1-^{14}C]glycine in isolated rat liver mitochondria. The mitochondria were incubated as described under Materials and Methods under state 4 conditions in the absence of any additional substrate (●) and in the presence of 20 mM L-carnitine (○), 5 mM propionate (▲) and 5 mM propionate plus 20 mM L-carnitine (△). The glycine concentration was 5 mM in all cases.

under the conditions of these experiments, the fatty acids may become a significant source of reducing equivalents. Finally, there have been a variety of nonspecific effects of longer chain acyl-CoA derivatives on various enzymatic processes reported. On the basis of these results, it is clear that the stimulation of mitochondrial glycine decarboxylation by propionate is not specific for propionate but is common to all of the short-chain fatty acids which were tested. Similar results have been observed with the medium chain length fatty acid octanoate (data not shown).

Activation of the fatty acid by an appropriate acyl-CoA synthetase to its respective acyl-CoA derivative is the only common reaction shared by all fatty acids. Hence, we have focused our attention upon this reaction. Propionate was chosen for detailed study because (a) it evokes an appreciable stimulation of glycine decarboxylation by rat liver mitochondria and (b) we have the capability in our laboratory to measure all of the intermediates of propionate metabolism including all nucleotides and acyl-CoA derivatives.

The apparent lack of specificity of this effect of fatty acids stimulating glycine decarboxylation suggests that one or more fatty acyl-CoA intermediates are not responsible for oxidizing the NADP(H) redox couple and/or stimulating glycine decarboxylation. In order to substantiate that an acyl-CoA derivative is not involved directly in this effect, we measured the production of $^{14}CO_2$ from [1-^{14}C]glycine by rat liver mitochondria in the absence and presence of propionate and/or L-carnitine (Figure 2). Addition of L-carnitine to mitochondrial incubations diminishes the level of the acyl-CoA derivatives, regenerating free CoASH (Batenburg & Olson, 1976; Patel et al., 1983), by converting the acyl-CoA to the respective acylcarnitine derivative by an appropriate acyl-CoA:carnitine acyltransferase. The addition of L-carnitine to the mitochondrial incubations did not abrogate the propionate-mediated stimulation of mitochondrial glycine decarboxylation but even stimulated glycine decarboxylation further. Addition of L-carnitine in the absence of propionate inhibited glycine decarboxylation slightly. To verify the results of this experiment, the intramitochondrial contents of propionyl-CoA, NADH, and NADPH were measured under the incubation conditions in Figure 2, and the results are presented in Table II. As predicted, in the presence of L-carnitine (20 mM), only

Table II: Effect of Carnitine on the Intramitochondrial Content of Propionyl-CoA, NADH, and NADPH in the Absence and Presence of Propionate[a]

	content (nmol·mg of mitochondrial protein^{-1})		
incubation conditions	propionyl-CoA	NADH	NADPH
no addition	trace	0.28 ± 0.02	4.08 ± 0.36
carnitine, 20 mM	trace	0.21 ± 0.07	4.63 ± 0.33
propionate, 5 mM	1.9 ± 0.36	0.04 ± 0.02	1.35 ± 0.12
propionate, 5 mM, + carnitine, 20 mM	0.10 ± 0.05	0.05 ± 0.02	1.16 ± 0.14

[a] Propionyl-CoA and the reduced pyridine nucleotides were extracted and quantitated as described under Materials and Methods. The mitochondria were incubated under state 4 conditions with 5 mM glycine. The values are means ± the SD for three independent determinations.

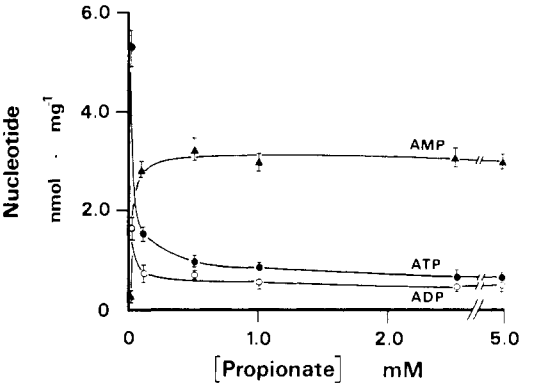

FIGURE 3: Effect of propionate concentration on the intramitochondrial content of ATP (●), ADP (○), and AMP (▲) in rat liver mitochondria. The mitochondria were incubated as described under Materials and Methods under state 4 conditions with 5 mM glycine. The mitochondrial incubations were extracted after 7.5 min and the adenine nucleotides analyzed by anion-exchange HPLC as described under Materials and Methods. The values represented in this figure are means ± SD for three separate determinations.

a trace of propionyl-CoA was formed upon the addition of propionate, but the propionate-induced decrease in the intramitochondrial NADPH level was not altered. In the presence of propionate, propionyl-CoA was by far the major acyl-CoA derivative observed. Only small amounts of methylmalonyl-CoA and succinyl-CoA were detected, and like propionyl-CoA, these intermediates were reduced markedly by L-carnitine (data not shown).

The above experiments suggest that neither CoASH nor an acyl-CoA derivative is involved directly in the oxidation of the NADP(H) redox couple and/or the stimulation of mitochondrial glycine decarboxylation by propionate. Other possible effectors of this enzymatic system are the various mitochondrial adenine nucleotides. In the process of fatty acid activation to the respective acyl-CoA derivative, there occurs a pyrophosphate cleavage of ATP to AMP and pyrophoshate. Depicted in Figure 3 are the levels of intramitochondrial ATP, ADP, and AMP in rat liver mitochondria, under state 4 conditions, as a function of the concentration of added propionate. Addition of propionate to the mitochondrial incubations elicited a substantial decrease in intramitochondrial ATP and a large increase in AMP. Also there occurred a decrease in intramitochondrial ADP but not to the same extent as was seen for the changes in ATP and AMP. As there is no strong reducing substrate present in these incubations, propionate lowered the phosphate potential of the mitochondrial system in a fashion similar to placing the mitochodria in state 3 or the uncoupled state, both conditions which stimulate mitochondrial glycine decarboxylation. When the mitochondrial respiratory chain is energized, the equilibrium of

Table III: Effects of Various Metabolic Conditions (Depicted in Figures 4 and 5) on the Mitochondrial Content of ATP, ADP, AMP, NADH, and NADPH in Rat Liver Mitochondria[a]

incubation conditions	content (nmol·mg of mitochondrial protein^{-1})				
	ATP	ADP	AMP	NADH	NADPH
no addition	5.04 ± 0.69	1.93 ± 0.18	1.35 ± 0.09	0.28 ± 0.02	4.08 ± 0.36
propionate, 5 mM	0.71 ± 0.28	0.76 ± 0.46	4.53 ± 0.91	0.04 ± 0.02	1.35 ± 0.12
oligomycin, 10 μg/mL	1.45 ± 0.21	5.08 ± 0.75	0.54 ± 0.14	0.32 ± 0.02	3.95 ± 0.46
propionate, 5 mM, + oligomycin, 10 μg/mL	0.21 ± 0.05	3.77 ± 0.23	1.96 ± 0.14	0.11 ± 0.03	1.20 ± 0.41
FCCP, 2.5 μM	0.52 ± 0.04	3.95 ± 0.59	0.83 ± 0.07	ND[b]	ND
α-ketoglutarate, 5 mM, + oligomycin, 10 μg/mL	6.64 ± 0.26	0.15 ± 0.04	1.01 ± 0.04	275 ± 0.36	5.65 ± 0.41
α-ketoglutarate, 5 mM, + oligomycin, 10 μg/mL, + FCCP, 2.5 μM	4.01 ± 0.52	1.59 ± 0.28	0.42 ± 0.10	ND	0.65 ± 0.12
α-ketoglutarate, 5 mM, + acetoacetate, 10 mM	5.33 ± 0.69	1.73 ± 0.21	0.48 ± 0.10	1.09 ± 0.08	5.61 ± 0.39
α-ketoglutarate, 5 mM, + acetoacetate, 10 mM, + propionate, 5 mM	3.17 ± 0.32	1.36 ± 0.36	1.75 ± 0.33	0.29 ± 0.08	4.32 ± 0.32
ATP, 10 mM				0.25 ± 0.09	3.26 ± 0.18
propionate, 5 mM, + ATP, 10 mM				0.13 ± 0.10	3.31 ± 0.31
FCCP, 2.5 μM, + oligomycin, 10 μg/mL, + ATP, 10 mM				ND	0.35 ± 0.19

[a] The adenine nucleotides were extracted (at 7.5 min) and quantitated as described under Materials and Methods. The values represent the means ± the SD for three independent determinations. [b] ND, not detectable.

the energy-linked transhydrogenase ($NADH + NADP^+ \rightleftharpoons NAD^+ + NADPH$) is as high as 500, but in the absence of energy (i.e., a proton gradient), the equilibrium of the reaction is very near unity. Hence, when the mitochondrial respiratory chain is uncoupled or "deenergized", electron flow occurs rapidly from NADPH to NAD^+ through the energy-linked transhydrogenase (Lee & Ernster, 1965; van Dam & ter Welle, 1965).

Upon close examination, it was noted that the changes in the intramitochondrial adenine nucleotides occured at much lower concentrations of propionate than did the changes in intramitochondrial NADPH and/or glycine decarboxylation. This observation may be explained by the fact that the energy required to drive the energy-linked transhydrogenase at half-maximal rate is much lower than the energy required to drive half-maximal rates of oxidative phosphorylation (van Dam & ter Welle, 1965; Lee & Ernster, 1968). An alternative explanation is that short-chain fatty acids oxidize the NADP(H) redox couple by uncoupling the mitochondria, even though they may not be uncouplers in the classical sense. It has long been known that long-chain fatty acids are capable of uncoupling oxidative phosphorylation (Pressman & Lardy, 1956; Lehninger & Remmerg, 1959), but short-chain fatty acids do not appear to be very effective uncouplers. The addition of propionate (as high as 10 mM) to state 4 mitochondria respiring on glutamate (5 mM) plus malate (5 mM) or on α-ketoglutarate (5 mM) evoked no change in the rate of oxygen consumption (data not shown). Alternatively, initiation of state 3 (ADP, 0.2 mM; hexokinase, 7 IU/mL; glucose, 30 mM; magnesium chloride, 1 mM) or the addition of uncoupler (FCCP, 2.5 μM) resulted in a very rapid depletion of oxygen in the incubation. In addition, propionate did not relieve the inhibition of mitochondrial glycine decarboxylation which we observed previously (Hampson et al., 1983) upon addition of succinate, α-ketoglutarate, glutamate plus malate, and pyruvate plus malate, while initiation of state 3 partially released and uncouplers nearly totally relieved the inhibition of glycine decarboxylation (data not shown). However, it must be noted that uncoupling mitochondria is a relative process. According to the chemiosmotic coupling hypothesis, the

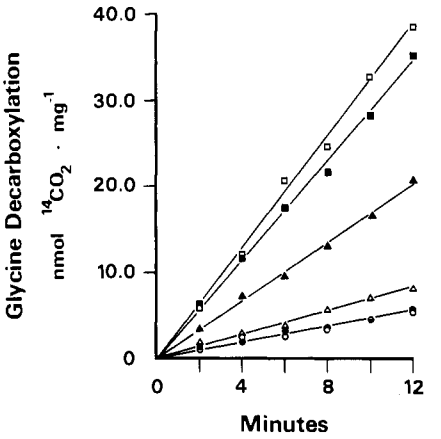

FIGURE 4: Effect of oligomycin on $^{14}CO_2$ production from [1-^{14}C]-glycine by rat liver mitochondria in the absence and presence of propionate or uncoupler. The mitochondria were incubated as described under Materials and Methods in the absence of any additional substrate (●) and in the presence of 10 μg/mL oligomycin (○), 5 mM propionate (▲), 5 mM propionate plus 10 μg/mL oligomycin (△), 2.5 μM FCCP (■), and 2.5 μM FCCP plus 10 μg/mL oligomycin (□). The [1-^{14}C]glycine concentration was 5 mM.

electron-transport chain continuously generates a proton gradient across the mitochondrial membrane, while an uncoupler causes the dissipation of this proton gradient. If the rate of dissipation of the proton gradient equals the rate of proton pumping, the mitochondrion becomes uncoupled. Under the incubation conditions employed in these experiments, there is no readily oxidizable substrate available to the mitochondria. Hence, the rate of proton pumping is relatively low, and the short-chain fatty acids may be capable of uncoupling the mitochondria under these conditions.

In an attempt to determine to what extent uncoupling of the mitochondria and the rapid consumption of intramitochondrial ATP each contribute to the oxidation of NADPH by propionate, the experiments presented in Figure 4 and a portion of Table III were performed. If propionate causes an oxidation of the NADP(H) redox couple by lowering the phosphate potential and, hence, deenergizing the electron-

transport chain, stimulation of mitochondrial glycine decarboxylation by propionate should be abrogated by the addition of oligomycin. Depicted in Figure 4 is the effect of oligomycin on the production of $^{14}CO_2$ from [1-^{14}C]glycine in the absence and presence of propionate and/or uncoupler. The addition of oligomycin (10 μg/mL) abolished nearly completely the stimulation of mitochondrial glycine decarboxylation by propionate with little or no change in the stimulation of decarboxylation by FCCP (2.5 μM). This result is consistent with the hypothesis that propionate oxidized the NADP(H) redox couple by initiating a rapid consumption of intramitochondrial ATP. However, when intramitochondrial NADP(H) and NAD(H) were assayed, it was observed that propionate still oxidized the NADP(H) redox couple in the presence of oligomycin, suggesting that propionate uncoupled the mitochondria. The noncorrelation, in this case between the oxidation–reduction state of the NADP(H) couple and mitochondrial glycine decarboxylation, is a cause for some concern, but it is quite likely that this result is an anomaly of the experimental conditions employed. A similar result was observed previously in this laboratory in studies of α-ketoglutarate oxidation in isolated rat liver mitochondria (Olson & Allgyer, 1972, 1973a,b). It was reported that addition of oligomycin to uncoupled mitochondria, respiring on α-ketoglutarate, caused a decrease in the rate of α-ketoglutarate oxidation, even though the mitochondrial pyridine nucleotides remained oxidized. Measurement of the oxidation–reduction state of the mitochondrial flavins by dual-wavelength and fluorescence spectroscopy indicated that one flavoprotein became reduced under these conditions. Indications were that this reduced flavoprotein was dihydrolipoyl hydrogenase, a component of the α-ketoglutarate complex and also a component of the glycine cleavage system. Hence, it is quite possible that a similar type of situation has occurred with oxidation of glycine by the glycine cleavage system. Several experimental results support this contention.

The fact that oligomycin did not prevent the stimulation of glycine decarboxylation by the uncoupler FCCP indicates that oligomycin is not a direct inhibitor of the glycine cleavage system. The reason oligomycin failed to prevent the stimulation of mitochondrial glycine decarboxylation by FCCP but prevented the propionate-induced stimulation is not obvious. If propionate oxidized the NADP(H) redox couple through an uncoupling mechanism, one would expect similar results with FCCP. It is possible that, under these conditions, both propionate and FCCP uncoupled the mitochondria to a great enough extent to oxidize the NADP(H) redox couple. However, the addition of uncoupler may have deenergized the electron-transport chain to a greater extent than propionate, a condition which may be critical for oligomycin to prevent the stimulation of mitochondrial glycine decarboxylation by propionate. This possibility is supported by the observation that FCCP stimulated mitochondrial glycine decarboxylation in the presence of propionate plus oligomycin (data not shown).

These observations indicate that propionate oxidized the NADP(H) redox couple by partially uncoupling the mitochondria. However, in the presence of oligomycin, there is no longer a source of ATP for the activation of propionate, and hence, propionate is no longer a substrate for the mitochondria. Neither propionyl-CoA nor any of its products were present in extracts of mitochondria incubated in the presence of propionate plus oligomycin (data not shown). In the absence of oligomycin, the combination of propionate and glycine oxidation may supply enough reducing equivalents such that propionate no longer effectively uncouples the mitochondria. The following observations support this contention. The intramitochondrial adenine nucleotide profile in the absence of propionate is quite different than the adenine nucleotide profile obtained with FCCP. In both cases, the intramitochondrial content of ATP is reduced drastically, but in the presence of propionate, the intramitochondrial AMP content was elevated while, with FCCP, the ADP content was elevated substantially. Moreover, when mitochondria were incubated with propionate plus oligomycin, the adenine nucleotide profile resembles much more closely the profile obtained upon incubation with FCCP. Furthermore, the observation that L-carnitine did not abrogate the stimulation of mitochondrial glycine decarboxylation by propionate, but actually stimulated slightly mitochondrial glycine decarboxylation, is consistent with this suggestion. Such an effect may be due to a mass-action effect of L-carnitine, removing a product of the reaction (i.e., propionyl-CoA), regenerating free CoASH, and increasing the rate of ATP utilization.

Hence, no conclusive statement can be made as to the exact mechanism by which propionate oxidizes the NADP(H) redox couple. Whether the NADP(H) couple is oxidized due to the rapid activation of propionate to propionyl-CoA or to a partial uncoupling of the mitochondrial respiratory chain, the end result is the same. Propionate lowers the phosphate potential of the mitochondrion, in turn increasing the rate of flow of electrons through the respiratory chain. In the absence of a strongly reducing substrate, this process leads to the flow of electrons from NADPH to NAD^+ via the energy-linked transhydrogenase.

The changes in the adenine nucleotide distribution observed upon the addition of propionate are consistent with the suggestion that fatty acid activation deenergizes the mitochondrial system, causing an oxidation of the NADP(H) redox couple. It is still conceivable that the oxidation of the NADP(H) redox couple may be related indirectly to the stimulation of mitochondrial glycine decarboxylation and that one or more of the adenine nucleotides is a direct effector of the glycine decarboxylation reaction. To assess this possibility, as depicted in Figure 5, mitochondrial incubations were performed under conditions which should oxidize the NADP(H) redox state yet maintain an elevated intramitochondrial ATP level. Depicted in Figure 5A are rates of production of $^{14}CO_2$ from [1-^{14}C]-glycine by state 4 or uncoupled rat liver mitochondria in the absence and presence of α-ketoglutarate (5 mM) plus oligomycin (10 μg/mL). α-Ketoglutarate plus oligomycin will maintain the intramitochondrial ATP content at an elevated level, even in the presence of uncoupler, through the combined action of the succinate thiokinase step of the tricarboxylic acid cycle, which produces GTP, and the nucleoside diphosphokinase reaction, which transfers the terminal high-energy phosphate from GTP to ADP, forming ATP. Oligomycin prevents the subsequent breakdown of ATP by the oligomycin-sensitive ATPase. Maintenace of an elevated intramitochondrial ATP content in this manner did not prevent the stimulation of mitochondrial glycine decarboxylation in the uncoupled state. These results were verified by measurements of the intramitochondrial adenine and reduced pyridine nucleotides contents which are presented as a portion of Table III. The addition of α-ketoglutarate plus oligomycin to uncoupled mitochondria maintained the intramitochondrial ATP content at an elevated level while the NADP(H) redox couple was oxidized nearly completely.

A similar type of experiment was performed with the stimulation of mitochondrial glycine decarboxylation by propionate. Again α-ketoglutarate was utilized to elevate the intramito-

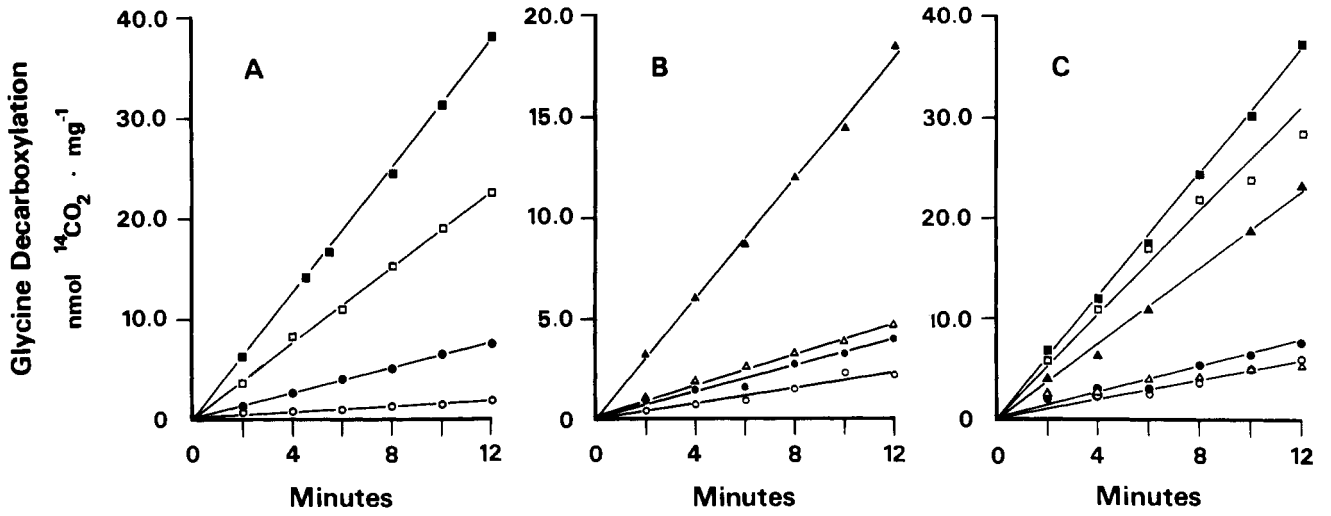

FIGURE 5: Effect of elevated intramitochondrial ATP levels under oxidizing conditions on $^{14}CO_2$ production from [1-^{14}C]glycine by rat liver mitochondria. (A) Effect of α-ketoglutarate plus oligomycin in the absence and presence of uncoupler: no addition (●); 5 mM α-ketoglutarate plus 10 μg/mL oligomycin (○); 2.5 μM FCCP (■); 2.5 μM FCCP plus 5 mM α-ketoglutarate plus 10 μg/mL oligomycin (□). (b) Effect of α-ketoglutarate plus acetoacetate in the absence and presence of propionate: no addition (●); 5 mM α-ketoglutarate plus 10 mM acetoacetate (○); 5 mM propionate (▲); 5 mM propionate plus 5 mM α-ketoglutarate plus 10 mM acetoacetate (△). (C) Effect of added ATP in the absence and presence of propionate or uncoupler: no addition (●); 10 mM ATP (○); 5 mM propionate (▲); 5 mM propionate plus 10 mM ATP (△); 2.5 μM FCCP (■); 2.5 μM FCCP plus 10 mM ATP (□). The mitochondrial incubations were performed as described under Materials and Methods with the concentration of [1-^{14}C]glycine at 5 mM.

chondrial ATP content. Acetoacetate (10 mM) was included in the incubations to consume NADH in the β-hydroxybutyrate dehydrogenase reaction. Oligomycin was not included in these incubations as the central point of the mechanism by which short-chain fatty acids oxidize the NADP(H) redox couple was to increase the rate of oxidative phosphorylation, which oligomycin inhibits. As depicted in Figure 5B, propionate stimulated production of $^{14}CO_2$ from [1-^{14}C]glycine by rat liver mitochondria in the presence of α-ketoglutarate (5 mM) plus acetoacetate (10 mM). Measurement of the contents of intramitochondrial adenine and reduced pyridine nucleotides, presented as a part of Table III, indicated that propionate oxidized the NAD(H) and NADP(H) redox couples in the presence of α-ketoglutarate plus acetoacetate while the intramitochondrial ATP content remained relatively elevated.

Finally, the intramitochondrial ATP content was altered by adding exogenous ATP to the mitochondrial incubations. Shown in Figure 5C is the effect of ATP (10 mM) on $^{14}CO_2$ production from [1-^{14}C]glycine by rat liver mitochondria in the absence and presence of FCCP or propionate. The addition of ATP (10 mM) plus oligomycin (10 μg/mL) had little effect on the stimulation of mitochondrial glycine decarboxylation by uncoupler and, as presented in Table III, did not prevent the oxidation of NADPH. The addition of ATP (10 mM) eliminated completely the stimulation of mitochondrial glycine decarboxylation by propionate. However, this result was expected as ATP, under appropriate conditions, is capable of driving electron transport in the reverse direction, energizing the energy-linked transhydrogenase (Danielson & Ernster, 1963; Lee & Ernster, 1966), preventing oxidation of the NADP(H) redox couple, and preventing the stimulation of glycine decarboxylation. Measurement of the intramitochondrial NADH and NADPH contents, presented in Table III, demonstrates that the addition of ATP prevented the oxidation of NADPH upon addition of propionate.

On the basis of the evidence presented in Figure 5 and Table III, the adenine nucleotides are not direct effectors in the stimulatory effects of uncoupler or propionate on mitochondrial glycine decarboxylation. No correlation was observed between mitochondrial glycine decarboxylation and ATP, ADP, or AMP while a correlation with the NAD(H) and the NADP(H) redox couples was maintained. All of these experiments were extremely repeatable, with rates of glycine decarboxylation varying no more than 10–15% from one mitochondrial preparation to another.

It should be noted that the observed stimulation of glycine decarboxylation by short-chain fatty acids in isolated rat liver mitochondria likely is not a physiologically relevant process which operates in vivo. The mechanism by which the short-chain fatty acids stimulate mitochondrial glycine decarboxylation as described above requires that there be no readily oxidizable substrate present which supplies reducing equivalents and thus prevents a significant lowering of the intramitochondrial phosphate potential. Intact tissues usually possess substrates available for mitochondrial processing (e.g., pyruvate, fatty acids, amino acids) along with reducing equivalents and/or phosphate potential generated in the cytosol. Moreover, in vivo, a significant portion of the activation of short-chain fatty acids occurs in the cytosol rather than in the mitochondria. However, the sequence of events leading to the stimulation of glycine decarboxylation by propionate in isolated mitochondria appears to be consistent with that observed in the intact liver. Studies reported from Lardy's laboratory (Blair et al., 1973) demonstrated that propionate in the perfused liver lowered slightly the ATP/ADP ratio and raised the intracellular AMP content.

Even though the effects of short-chain fatty acids on glycine cleavage activity in isolated rat liver mitochondria may not be physiologically significant, this effect has proven to be a valuable tool in deducing factors that regulate the rate of mitochondrial glycine catabolism. There are but a few methods for manipulating the NADP(H) redox state in isolated mitochondria short of uncoupling the mitochondria. However, studies of glycine catabolism in the isolated perfused rat liver (Hampson et al., 1983) indicate that the sensitivity of the meetabolic flux through the glycine cleavage system to the oxidation–reduction state of the mitochondria NAD(H) and NADP(H) redox couples occurs in vivo.

Another laboratory investigated the regulatory effects of propionate on glycine catabolism in isolated rat liver mitochondria and found that propionate inhibited production of

$^{14}CO_2$ from [1-^{14}C]glycine in direct contradiction to our observations (Ugarte et al., 1979; Benavides et al., 1980). However, upon examination of their reaction conditions, we noted that these investigators did not include inorganic phosphate in their incubation medium. Additionally, an ADP-regenerating system (i.e., glucose plus hexokinase) was included which certainly should have caused a complete depletion of the endogenous inorganic phosphate in this mitochondrial system. The depletion of inorganic phosphate would have a comparable effect to adding oligomycin to the incubations.

The difference between our results and those of Ugarte et al. (1979) and Benavides et al. (1980) stresses the importance of strictly controlling the metabolic state of the mitochondrial system when investigating the effects of a particular compound such as propionate on mitochondrial possesses (e.g., the glycine cleavage reaction).

A series of clinical disorders characterized by significant hyperglycinemia have been shown to be associated with abnormal glycine catabolism. There are two types of such hyperglycinemias, nonketotic [for a review, cf. Nyhan (1978)] and ketotic [for a review, cf. Tanaka (1975)]. The ketotic type of hyperglycinemia appears to be a secondary consequence of genetic disorders in various other metabolic pathways. Attempts to determine how abnormal glycine catabolism originates in these disorders have led several laboratories to investigate the effects of metabolites from various metabolic pathways, in which enzymatic defects are known to lead to hyperglycinemia, on glycine cleavage activity in isolated rat liver mitochondria (Ugarte et al., 1979; Kølvraa, 1979; Benavides et al., 1980; Mortenson et al., 1980) and in tissue homogenates (O'Brien, 1978; Mortenson et al., 1980). A variety of different postulates as to what causes hyperglycinemia have resulted from these studies. The extent to which the NADP(H) or NAD(H) oxidation–reduction couples influence the activity of the mitochondrial glycine cleavage system in any of these hyperglycinemic states is an open question at this time.

Registry No. NADPH, 53-57-6; NADH, 58-68-4; ATP, 56-65-5; 5′-AMP, 61-19-8; ADP, 58-64-0; acetic acid, 64-19-7; propionic acid, 79-09-4; butyric acid, 107-92-6; pentanoic acid, 109-52-4; glycine, 56-40-6.

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