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carnitine
citric acid cycle
metabolism

propionyl CoA
succinate:CoA ligase

Propionate Inhibition of Succinate:CoA Ligase (GDP) and the Citric Acid Cycle in Mitochondria

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Summary

Propionate inhibits oxygen consumption by rat liver mitochondria when glutamate, α-ketaglutarate, and succinate are substrates. Carnitine prevents this effect. The pattern of inhibition of $^{14}CO_2$ release from metabolic intermediates indicates citric acid cycle inhibition between succinate:coenzyme A (CoA) ligase (GDP) and malate dehydrogenase. Propionyl CoA is synthesized from propionate in mitochondria. Propionyl CoA is a potent inhibitor of succinate:CoA ligase with positive cooperativity and half-maximal inhibition at 2×10^{-4} M propionyl CoA.

Speculation

Inhibition of oxidative phosphorylation and the citric acid cycle may produce the Reye's-like syndrome which occurs in propionic acidemia and possibly related organic acid or mitochondrial disorders. Mitochondrial acyl coenzyme A may be increased in these patients and respond favorably to carnitine therapy.

Propionic acidemia is a rare inborn error of lipid and amino acid catabolism (21). It occasionally produces a toxic encephalopathy resembling Reye's syndrome (26). Reye's syndrome probably results from disrupted mitochondrial metabolism (5). Similar metabolic abnormalities occur in propionic acidemia and mitochondrial disorders (26), including hyperammonemia, lactic acidosis, hypoglycemia, and ketosis. Understanding the effects of propionic acid on mitochondrial metabolism might, therefore, clarify the pathophysiology of the associated abnormalities in propionic acidemia and be relevant to Reye's syndrome and other mitochondrial disorders.

METHODS

Mitochondria were prepared from rat liver and polarographic assays performed as previously described (27). Propionate was preincubated with mitochondria for 4.5 min at 30°C before addition of other reagents; controls were preincubated without propionate. A final propionate concentration of 4.76 mM was used in standard assays. ^{14}C-Labeled compounds were added (final concentration, 3.3 mM) after preincubation as above with additional incubation at 30°C for 15 min and $^{14}CO_2$ collection as previously reported (28). For the decarboxylation studies, the polarographic assay mixture was supplemented with ADP (final concentration, 3.3 μM), glucose (final concentration, 33 μM), and yeast hexokinase (Sigma Chemical Co., St. Louis, MO; 1.9 IU/0.3 ml assay volume) to maintain state 3 rates during incubation. Ascorbate and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine were used as previously described (30). Succinate:coenzyme A (CoA) ligase (GDP) (EC 6.2.1.4) assays had in final concentrations: 0.1 mM GDP, 0.1 mM succinyl CoA, and 10 mM $MgCl_2$ in 100 mM phosphate buffer (pH 7.4). Assays for the reverse reaction contained 0.1 mM GTP, 0.1 mM CoA, 10 mM $MgCl_2$, and 10 mM succinate in 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4). Thioester concentration was followed at 235 nm with a Beckman model 25 recording spectrophotometer at 30°C during the initial several min when linearity with time was observed. Enzyme concentration was adjusted to maintain initial rates of less than 0.04 A units/min. The reaction was initiated by addition of enzyme after a stable, flat baseline was obtained. The difference of molar absorptivities between succinyl CoA and CoA under these conditions is 4.0 mM cm (4). Purified pig heart succinate:CoA ligase (SCL) was purchased from Sigma Chemical Co. for use in the kinetic analyses. Statistical and Michaelis-Menton enzyme kinetic analyses match those used previously (29). Hill plots (7) were evaluated by linear regression analysis. Propionic acid was neutralized with NaOH or KOH before use in all these experiments.

RESULTS

Propionate inhibited state 3 rates and slightly stimulated state 4 rates of oxygen consumption (Table 1). Both these effects contributed to a decreased respiratory control ratio (RCR) (Table 2). These effects were observed with glutamate, α-ketoglutarate, and succinate. There was no alteration of ADP:O ratios, despite the reduced RCR (Table 2). Carnitine, when preincubated with propionate, prevented or minimized these effects of propionate. This carnitine effect was variable and sometimes only seen at lower propionate concentrations. Carnitine did not reverse the effects of propionate in the short time (about 1 min) available after assessing the primary effect of propionate. These effects of propionate are illustrated in a representative set of experiments (Fig. 1). Propionate effects on other substrates suitable for polarographic assays, including pyruvate:malate and palmitylcarnitine, could not be as accurately assessed because of the poor RCR's that occurred in control runs after the 5 min preincubation.

Preincubation of mitochondria with propionate was necessary for consistent, maximal inhibition of state 3 rates. Following a preincubation of mitochondria and maximal inhibition of state 3 rates, subsequently added mitochondria are not immediately affected. There was a variation in the susceptibility of mitochondria. The relatively high propionate concentration was used because all preparations were affected at this level. Some mitochondrial preparations were affected at propionate concentrations as low as 0.4 mM.

Carbon dioxide release from [1-^{14}C]pyruvate, [2-^{14}C]pyruvate, [6-^{14}C]citrate, and [1-^{14}C]α-ketoglutarate was not significantly inhibited by propionate. In contrast, $^{14}CO_2$ release from [1,4-^{14}C]succinate, [*U*-^{14}C]glutamate, and [*U*-^{14}C]malate was inhibited by propionate (Table 3).

SCL was inhibited by propionyl CoA. Inhibition at low propionyl CoA concentrations appeared to be simple noncompetitive on 1/s *versus* 1/v plots with K_I of 0.1 to 0.3 mM. At higher

Table 1. *Effect of propionate on state 3, state 4, and N,N,N',N'-tetramethyl-p-phenylenediamine: ascorbate rates of oxygen consumption (nA O/min/mg protein)*

Substrate (3.3 mM)	State 3		State 4	
	Control	Propionate (4.76 mM)	Control	Propionate (4.76 mM)
Glutamate	71.5 ± 4.9[1] (11)[2]	38.2 ± 7.1 (11)[3]	12.5 ± 1.1 (11)	17.9 ± 1.2 (11)[4]
α-Ketoglutarate	54.3 ± 10.5 (6)	24.4 ± 2.2 (6)[3]	14.2 ± 1.6 (6)	21.5 ± 3.3 (6)[4]
Succinate	148.0 ± 14.0 (6)	100.9 ± 15.4 (6)[3]	26.6 ± 2.3 (6)	37.9 ± 6.6 (6)[4]
N,N,N',N'-Tetramethyl-*p*-phenylenediamine: ascorbate	36.4 ± 5.1 (6)	43.8 ± 6.6 (5)		

[1] Mean ± S.E.
[2] Numbers in parentheses, *n*.
[3] Significantly less than control; *t* test (paired samples); $P < 0.01$.
[4] Significantly greater than control; *t* test (paired samples); $P < 0.05$.

Table 2. *Effect of propionate on oxidative phosphorylation*

Substrate (3.3 mM)	Respiratory control ratios		ADP:O ratios	
	Control	Propionate (4.76 mM)	Control	Propionate[1] (4.76 mM)
Glutamate	6.4 ± 0.5[2] (11)[3]	2.1 ± 0.3 (11)[4]	2.9 ± 0.2 (11)	3.4 ± 0.3 (8)
	[4.3–9.3][5]	[1.0–4.0]	[2.2–5.0]	[2.3–4.7]
α-Ketoglutarate	3.8 ± 0.5 (6)	1.2 ± 0.1 (8)[4]	3.2 ± 0.4 (6)	[6]
	[2.5–5.5]	[1.0–2.0]	[2.0–4.4]	
Succinate	5.6 ± 0.3 (6)	3.4 ± 0.5 (5)[4]	2.3 ± 0.3 (6)	2.3 ± 0.3 (5)
	[4.6–6.5]	[2.1–4.7]	[1.6–2.5]	[1.6–3.1]

[1] No significant difference with propionate.
[2] Mean ± S.E.
[3] Numbers in parentheses, *n*.
[4] $P < 0.005$ by Mann-Whitney (Wilcoxin) statistic.
[5] Numbers in brackets, range.
[6] Unable to evaluate because of poor state 3 → 4 transition.

propionyl CoA concentrations, this plot was concave upward, indicating a different mechanism of inhibition. The Dixon plot (i *versus* 1/v) was also nonlinear (Fig. 2). The Hill plots were linear (Fig. 3) with a Hill coefficient (*n*) of 3.29 ± 0.26 (mean ± S.E.; 6 determinations) and half-maximal inhibition at 246 ± 27 μM (calculated from $\log \frac{V_i}{V_0 - V_i}$ = O intercept). Propionic acid and propionyl CoA were not substrates for the enzyme when added to the reaction mixture in place of succinate or succinyl CoA, nor did propionate affect enzyme activity. Typical Michaelis-Menton kinetics were obtained in the absence of propionyl CoA with a succinyl CoA K_m of 23 μM and succinate K_m of 313 μM in the forward and reserve reactions, respectively.

Several other acyl CoA derivatives were evaluated for similar effects on SCL. These limited experiments, one or 2 Hill plots per compound, are included for completeness (Table 4) but must be viewed with caution until more extensive investigations are available.

DISCUSSION

Propionyl CoA inhibition of SCL may explain the impaired oxidative metabolism produced by propionate. Many features of propionic acidemia may relate to this effect. Levels of this inhibitor are probably reduced by carnitine *in vitro*, suggesting a clinical therapeutic modality.

Maximal effects occur with preincubation, implying inhibition by a metabolic product or depletion of vital compounds. Inhibitor is in the matrix rather than the incubation media because subsequently added mitochondria are unaffected. Addition of propionate to mitochondria increases propionyl CoA and reduces free CoA and acetyl CoA (25).

$^{14}CO_2$ release from [1-^{14}C]pyruvate, [2-^{14}C]pyruvate, [6-^{14}C]citrate, and [1-^{14}C]α-ketoglutarate occurs between the pyruvate dehydrogenase complex (PDHC) and succinyl CoA formation and is not inhibited by propionate. Complete release of $^{14}CO_2$ from [1,4-^{14}C]succinate, [*U*-^{14}C]glutamate, and [*U*-^{14}C]malate requires more than one turn through the citric acid cycle, and decarboxylation of these compounds is inhibited by propionate. This indicates that inhibition is between SCL and malate dehydrogenase (EC 1.1.1.37).

Propionyl CoA is an inhibitor of SCL with positive cooperativity. The Hill coefficient suggests 4 propionyl CoA-binding sites per mole of enzyme. Cooperativity can be due to allosteric interactions or, particularly with multisubstrate enzymes, to other mechanisms (7). No allosteric regulation was recognized in other studies, but they did not include propionyl CoA (17).

Other acyl CoA derivates are approximately equipollent in inhibiting SCL. The concentrations required are similar to those affecting pyruvate carboxylase (EC 6.4.1.1) (24), succinyl CoA:3 oxoacid transferase (EC 2.8.3.5) (6), carbamylphosphate synthetase (9), and the glycine cleavage system (15).

Free CoA also affects SCL. Hill coefficients and half-maximal inhibition concentrations are such that at low concentrations CoA has a modest effect, and propionyl CoA is without effect. At higher concentrations, propionyl CoA is a more potent inhibitor than an equivalent amount of CoA. Thus, if total CoA (free plus propionyl) is constant and the effects are additive, SCL is inhibited as the proportion of CoA in the propionyl CoA form is increased.

High acyl CoA levels may regulate citric acid cycle flux. Positive cooperativity optimizes regulatory potential by providing a "chemical switch" that turns off an enzyme at a critical inhibitor concentration. In patients with organic acidemias, this can result in catastrophic effects when high acyl CoA levels are the result of their metabolic block rather than reflecting their overall metabolic status.

Propionyl CoA inhibits the PDHC (2), and propionate inhibits state 3 rates of oxygen consumption with pyruvate as substrate (34). This oxygen consumption depends not only on NADH generated by the PDHC but, to a large degree, on subsequent

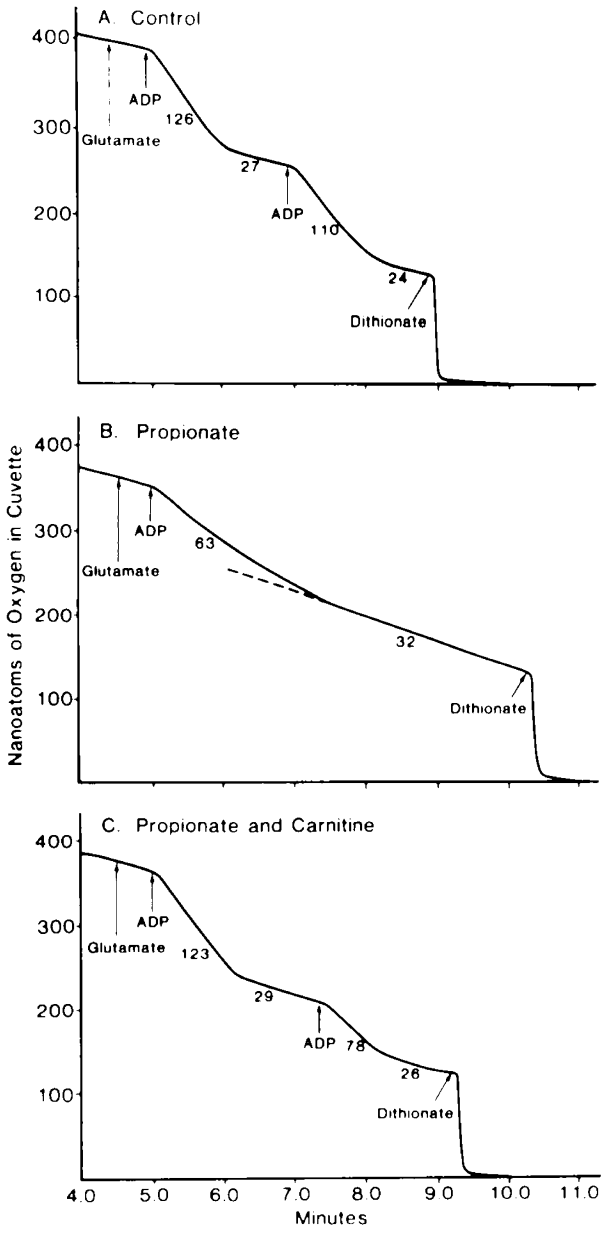

Fig. 1. Effect of propionate on oxygen consumption. Mitochondria were preincubated for 4.5 min with no additions (*A*), with 4.76 mM propionate (*B*), or with 4.76 mM propionate:5 mM carnitine (*C*). Propionate inhibited the state 3 rate and lowered the RCR. Carnitine prevented this effect. *Numbers below lines*, oxygen consumption in nA/min/mg protein.

oxidative reactions in the citric acid cycle. We observed no effect on [1-^{14}C]pyruvate decarboxylation although we used concentrations of propionate sufficient to inhibit oxygen consumption with pyruvate (34). Also, decarboxylation of [2-^{14}C]pyruvate via acetyl CoA was not affected, indicating that reduced oxygen consumption is not due to depletion of CoA or inhibition of the PDHC.

Serum propionate levels reach 5 mM in propionic acidemia (21). Infusion of propionate at this concentration produces tissue levels of about 50 μM propionyl CoA (20, 25). Mitochondrial levels are probably higher, particularly in the human disorder where propionate is derived from mitochondrial propionyl CoA. Concentrations used in our studies approximate those encountered in clinical situations.

Inhibition of the citric acid cycle would impair energy generation and the catabolism of many substrates. Propionate inhibits ureagenesis (8), and hyperammonemia might result from an energy deficiency state (30) or the inhibition of carbamylphosphate synthetase by propionyl CoA (9). Impaired flux through the citric acid cycle might contribute to the lactic acidosis and ketosis because catabolism of these compounds requires the citric acid cycle.

Table 3. *The effect of propionate on the decarboxylation of metabolic intermediates*[1]

Compound (3.3 mM)	Control	Propionate (4.76 mM)
[1-^{14}C]Pyruvate	10.30 ± 2.01[2] (8)[3]	8.01 ± 1.50 (8)
[2-^{14}C]Pyruvate	0.47 ± 0.13 (7)	0.34 ± 0.12 (7)
[*U*-^{14}C]Palmitate	0.48 ± 0.13 (6)	0.35 ± 0.08 (6)
[6-^{14}C]Citrate	2.39 ± 0.38 (6)	2.41 ± 0.43 (6)
[1-^{14}C]α-Ketoglutarate	8.00 ± 1.84 (5)	5.44 ± 1.10 (5)
[*U*-^{14}C]Glutamate	3.13 ± 0.50 (8)	1.17 ± 0.16 (8)[4]
[1,4-^{14}C]Succinate	3.43 ± 0.64 (7)	1.68 ± 0.38 (7)[4]
[*U*-^{14}C]Malate	0.98 ± 0.19 (5)	0.28 ± 0.03 (4)[5]

[1] nmoles/min/mg protein.
[2] Mean ± S.E.
[3] Numbers in parentheses, *n*.
[4] $P < 0.02$; *t* test, unpaired samples.
[5] $P < 0.01$; Mann-Whitney (Wilcoxin) statistic.

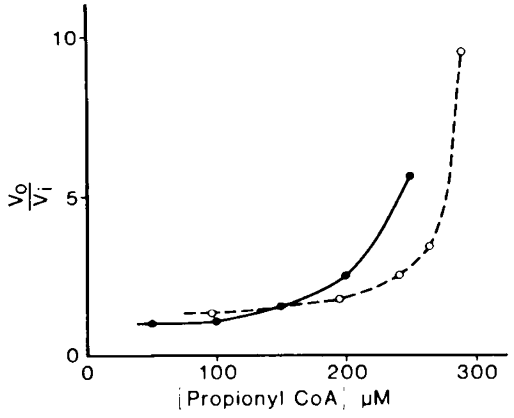

Fig. 2. Dixon plot. Positive cooperativity between inhibitor molecules is demonstrated. Effect of propionyl CoA on succinyl CoA formation (●) and disappearance (○) is shown.

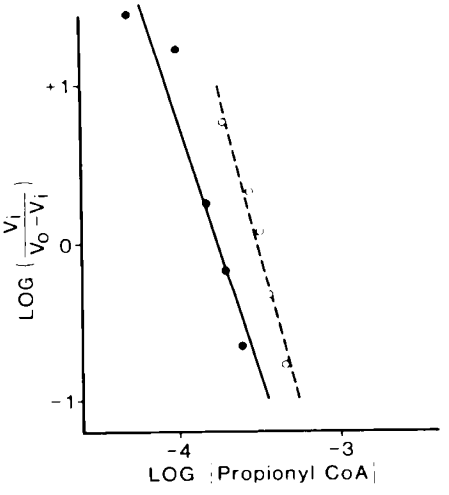

Fig. 3. Hill plot for propionyl CoA inhibition of succinate:CoA ligase (GDP). Linearity is observed in measuring the effect of propionyl CoA on succinyl CoA formation (●) and disappearance (○). Data are plotted according to the relationship: $\log \frac{V_i}{V_0 - V_i} = \log K - n \log$ [propionyl CoA], where V_0 is the activity without inhibitor, V_i the activity with propionyl CoA, and *n* is the Hill coefficient.

Table 4. *Acyl CoA inhibition of succinate:CoA ligase*[1]

Compound	Half-maximal inhibition (μM)[2]	Hill coefficient
Propionyl CoA	246 ± 27[3] (6)[4]	3.29 ± 0.26 (6)
Acetyl CoA	272, 283	3.50, 2.71
Tiglyl CoA	114, 113	2.02, 1.16
Methylmalonyl CoA	303	3.52
Glutaryl CoA	264	3.21
Butyryl CoA	274	3.21
Crotonyl CoA	173	2.26
Free CoA	349 ± 16 (4)	2.27 ± 0.12 (4)

[1] None of the acyl CoA compounds were substrates. Assay at saturating (97 μM) succinyl CoA and following its disappearance.

[2] Hill plot $\log\left(\frac{V_i}{V_0 - V_i}\right)$ = O intercept, calculated by linear regression analysis.

[3] Mean ± S.E.

[4] Numbers in parentheses, *n*.

Hyperglycinemia in propionic acidemia may result from inhibition of the glycine cleavage system (11, 31). Alternatively, acyl CoA derivatives may affect the succinate-glycine cycle. This proposed cycle remains poorly defined. δ-Aminolevulinic acid synthetase initiates the cycle; succinyl CoA and glycine condense, releasing CO_2. Subsequent reactions form γ,δ-dioxovaleric acid and α-ketoglutaraldehyde (14, 19). It is not clear whether succinyl CoA is reformed from succinate (via SCL) or from α-ketoglutarate (via its dehydrogenase). If the former is the case, then our results may provide an explanation for the hyperglycinemia in propionic acidemia. Our results may also have significance to patients with acute intermittent porphyria because crises are related to increased δ-aminolevulinic acid synthetase activity and changes in γ,δ-dioxovalerate excretion (14).

Fatty liver in propionic acidemia indicates impaired catabolism of fatty acids. Inhibition of palmitate decarboxylation in our study did not reach statistical significance, but did in another investigation (8). Low concentrations of propionate (0.5 mM) reduced acetyl CoA and acetyl carnitine formation apparently by inhibiting fatty acid activation or β-oxidation because propionate does not inhibit acetyl CoA synthetase (EC 6.2.1.1), deplete available carnitine, or accelerate acetyl CoA removal by citrate synthase (EC 4.1.3.7) at these low concentrations (20). Activation of fatty acids occurs by three mechanisms. High propionate concentrations may impair activation outside the matrix to a carnitine derivative by sequestering carnitine as a propionyl derivative. Activation also occurs in the matrix by either an ATP- or GTP-dependent acyl CoA synthetase (EC 6.2.1.3). The ATP-dependent acyl CoA synthetase produces AMP, and the only mechanism for converting AMP back to ADP in the matrix is by the GTP-AMP transphosphorylase (EC 2.7.4.10) (10). Therefore, both matrix-activating enzymes rely on GTP. The major sources of matrix GTP are SCL and ATP:nucleosidediphosphate phosphotransferase (EC 2.7.4.6) (16). Thus, oxidation of citric acid cycle substrates and fatty acids are mutually controlled (22). Inhibition of SCL by propionyl CoA could reduce matrix GTP and fatty acid activation. Low matrix GTP could contribute to the fatty liver in propionic acidemia. Low matrix GTP could also impair gluconeogenesis because the GTP-dependent gluconeogenic enzyme phosphoenolpyruvate carboxykinase (EC 4.1.1.32) is located in the mitochondrial matrix in man.

Propionyl CoA can be readily converted to propionylcarnitine in liver (1). At high propionate concentrations (4 mM in infusates), free carnitine is reduced to 10% of control in liver (20). This might account for the protective effect of added carnitine in our system. This observation might be relevant to the treatment of patients with propionic acidemia. Carnitine has been administered safely in other clinical situations (13, 23) and might ameliorate symptoms in propionic acidemia by reducing levels of toxic propionyl CoA. Carnitine may prevent the adverse metabolic effects of othe organic acids (3, 12).

Our results may be important to other organic acid disorder including methylmalonic aciduria, β-ketothiolase deficiency, an glutaric aciduria. Also, levels of short- and medium-chain organi acids are significantly elevated in serum of Reye's syndrom patients (18, 32, 33). Future studies of these disorders migh include assays of acyl CoA tissue and mitochondrial concentra tions. Direct assay of SCL may not be useful because acyl CoA derivatives would be diluted in preparing tissues for SCL assay.

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