Effects of propionate and carnitine on the hepatic oxidation of short- and medium-chain-length fatty acids

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Accumulation of propionate, or its metabolic product propionyl-CoA, can disrupt normal cellular metabolism. The present study examined the effects of propionate, or propionyl-CoA generated during the oxidation of odd-chain-length fatty acids, on hepatic oxidation of short- and medium-chain-length fatty acids. In isolated hepatocytes, ketone-body formation from odd-chain-length fatty acids was slow as compared with even-chain-length fatty acid substrates, and increased as the carbon chain length was increased from five to seven to nine. In contrast, rates of ketogenesis from butyrate, hexanoate and octanoate were all approximately equal. Propionate (10 mM) inhibited ketogenesis from butyrate, hexanoate and octanoate by 81 %, 53 % and 18 % respectively. Addition of carnitine had no effect on ketogenesis from the even-chain-length fatty acids, but increased the rate of ketone-body formation from pentanoate (by 53 %), heptanoate (by 28 %) and from butyrate or hexanoate in the presence of propionate. The inhibitory effect of propionate could not be explained by shunting acetyl-CoA into the tricarboxylic acid cycle, as CO₂ formation from butyrate was also decreased by propionate. Examination of the hepatocyte CoA pool during oxidation of butyrate demonstrated that addition of propionate decreased acetyl-CoA and CoA as propionyl-CoA accumulated. Addition of carnitine decreased propionyl-CoA by 50 % (associated with production of propionylcarnitine) and increased acetyl-CoA and CoA. Similar changes in the CoA pool were seen during the oxidation of pentanoate. These results demonstrate that accumulation of propionyl-CoA results in inhibition of short-chain fatty acid oxidation. Carnitine can partially reverse this inhibition. Changes in the hepatocyte CoA pool are consistent with carnitine acting by generating propionylcarnitine, thereby decreasing propionyl-CoA and increasing availability of free CoA. The data provide further evidence of the potential cellular toxicity from organic acid accretion, and supports the concept that carnitine's interaction with the cellular CoA pool can have a beneficial effect on cellular metabolism and function under conditions of unusual organic acid accumulation.

INTRODUCTION

Carnitine is known to be an obligate for the mitochondrial oxidation of long-chain fatty acids [1,2]. Recently, carnitine has been suggested to play an important role in maintaining cellular metabolism in the presence of accumulating organic acids. The accumulation of organic acids is often associated with the build-up of acyl-CoAs corresponding to the organic acids. The increased acyl-CoA concentration may disrupt normal metabolism by sequestering the cellular CoA pool or by direct inhibition of key enzymes. Under these conditions, carnitine can remove the accumulating acyl groups and liberate CoA by forming the corresponding acylcarnitine.

The metabolic toxicity associated with organic acids is illustrated by propionate. Propionate or propionyl-CoA can inhibit a number of important enzymes involved in cellular metabolism [3–6]. Propionate can inhibit the oxidation of a variety of substrates in hepatocytes [7], perfused liver [8] or isolated mitochondria [4,5,9]. Clinically, much of the symptomatology in propionicacidaemia may be the result of the disruption of normal

metabolism by propionyl-CoA [10]. *In vitro*, addition of carnitine can partially reverse the inhibitory effects of propionate on oxidative metabolism [7,11]. This effect of carnitine is associated with the production of propionylcarnitine from the accumulating propionyl-CoA [7]. *In vivo*, administration of carnitine can improve the clinical status of patients with propionicacidaemia [12,13] and other organic acidaemias [14–18].

Propionyl-CoA is formed during the terminal β -oxidation of odd-chain-length fatty acids. Propionate inhibits the β -oxidation of palmitate in isolated rat hepatocytes [7] and ketogenesis both *in vivo* [19] and *in vitro* [7,20]. The present studies were designed to characterize the effects of propionate and carnitine on the metabolism of even- and odd-chain-length fatty acids with total carbon chain length less than ten. The results demonstrate that propionyl-CoA dramatically inhibits the normal oxidation of short-chain fatty acids, and this effect is partially reversed by carnitine. Thus the interactions of propionate and carnitine metabolism can alter the hepatic oxidation of short- and medium-chain-length fatty acids.

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MATERIALS AND METHODS

Animals and hepatocyte isolation

Male Sprague-Dawley rats were used in all studies. Rats were allowed Wayne rat chow (Agway Co.) and water *ad libitum*. Food was removed from fasted rats 48 h before hepatocyte preparation. Preparation of cells from fed animals was begun between 07:00 and 08:00 h. Fed rats used in the present studies weighed 322 ± 8 g (mean $\pm$ S.E.M., $n = 49$). Fasted rats had initial and final body weights of 385 ± 27 g and 341 ± 28 g respectively (mean $\pm$ S.E.M., $n = 5$).

Isolation of hepatocytes was conducted by a modification of the collagenase perfusion technique of Berry & Friend [21], as previously described [22]. Hepatocyte viability averaged $92 \pm 1\%$ (mean $\pm$ S.E.M., $n = 54$) by Trypan Blue exclusion, and in all preparations was above 85%. Cells prepared from fed rats averaged 2.92 ± 0.12 mg of protein/ 10^6 cells (mean $\pm$ S.E.M., $n = 45$) and 16.6 ± 0.7 mg wet wt./ 10^6 cells (mean $\pm$ S.E.M., $n = 38$). Hepatocytes isolated from fasted rats averaged 1.88 ± 0.13 mg of protein/ 10^6 cells (mean $\pm$ S.E.M., $n = 5$) and had a wet weight of 8.0 mg/ 10^6 cells (mean of two preparations).

Incubations

Incubations were conducted in 25 ml Erlenmeyer flasks at 37 °C under an atmosphere of O₂/CO₂ (19:1). Cells at densities indicated in each experiment were preincubated for 30 min under the above conditions. Final composition of the incubations was 1% defatted bovine serum albumin, 134 mM-NaCl, 4.2 mM-KCl, 0.72 mM-MgSO₄, 0.9 mM-CaCl₂, 2.4 mM-Na₂HPO₄, 4.0 mM-glucose and 8.0 mM-Tris, pH 7.4. When included in an experiment, carnitine, propionate or α -oxobutyrate was added before the preincubation.

At the end of the preincubation (zero time), substrate was added to the flask. Samples for analysis of acetoacetate, β -hydroxybutyrate, acylcarnitines or acyl-CoAs were removed at the times indicated and added to HClO₄ to terminate the reaction. Production of ¹⁴CO₂ was monitored from radiolabelled substrates by using flasks equipped with side arms and centre wells. At the termination of the incubation, HClO₄ was added through the side arm to a final concentration of 3%, and ethanolamine/methoxyethanol (1:2, v/v; 0.3 ml) was added to the centre well to collect the ¹⁴CO₂ as previously described [7].

Assays

The CoA pool was assessed by a series of methods to permit components of the pool to be accurately quantified. Acetyl-CoA was measured by the end-point enzymic assay described by Decker [23], with fluorimetric detection of the NADH generated. Incubations contained 500 μ l of sample (3%-HClO₄ supernatant neutralized with K₂CO₃), 665 μ l of water, 800 μ l of 0.5 M-Tris (pH 8.1), 10 μ l of 0.1 M-malate, 5 μ l of NAD⁺ stock (7.4 mg of NAD⁺ in 950 μ l of water plus 50 μ l of 1 M-KHCO₃), and 12.0 units of malate dehydrogenase. At zero time, the fluorescence was recorded and 1.1 units of citrate synthase were added. The change in fluorescence at completion was noted, and the change in blank (sample replaced with water) incubations subtracted. A large amount of quenching was observed in many of the hepatocyte-derived samples. To permit accurate quanti-

fication, 2 nmol of acetyl-CoA was added to the sample after the initial change in fluorescence had stabilized. The acetyl-CoA content of the sample was then calculated from the change in fluorescence observed with the 2 nmol of acetyl-CoA internal standard.

Propionyl-CoA and butyryl-CoA were analysed by the h.p.l.c. method of Corkey *et al.* [24]. Samples (50–100 μ l of the neutralized 3%-HClO₄ supernatant) were injected on to an h.p.l.c. system with an U6K injector, 6000 series pump, model 441 detector and a Nova-Pak C₁₈ column (Waters Associates, Milford, MA, U.S.A.). The mobile phase was methanol/50 mM-KH₂PO₄ (pH 5.3) (1:6, v/v). Eluent A₂₅₄ was monitored and peaks were quantified by using a Hewlett-Packard 3390A integrator. Each sample was analysed twice, with the second injection containing 0.25 nmol each of propionyl-CoA and butyryl-CoA. The difference in peak areas between the two injections represented the internal standard from which the content of the acyl-CoAs in the original sample could be calculated.

The enzymic recycling assay described by Allred & Guy [25] was used to quantify the sum of CoA and acetyl-CoA in a neutralized sample of the HClO₄ supernatant. Final incubations contained 10 μ l of standard or sample, 328 μ l of water, 1.5 ml of triethanolamine (0.2 M)/malate (15 mM) stock solution (pH 8.0), 50 μ l of 0.02 M-dithiothreitol, 200 μ l of NAD⁺ (16 mM)/acetyl phosphate (54 mM) stock solution, 60 units of malate dehydrogenase, 15 units of phosphotransacetylase and 3.3 units of citrate synthase.

Acetoacetate and β -hydroxybutyrate were measured by the method of Olsen [26]. Samples of the incubation were removed at zero time and the times indicated and added to an equal volume of 5.1% HClO₄. The supernatant was then analysed for the ketone bodies, and net production was obtained by subtracting the concentration in the incubation at zero time. Identical incubations were also run in parallel, from which exogenous substrate was omitted. These values were also subtracted from experimental incubations to correct for generation of product from endogenous substrates under the conditions of interest. Analysis of acylcarnitines by fast-atom-bombardment mass spectroscopy was performed as previously described [7]. Protein concentrations were determined by a biuret method [27].

Data analysis

The generation of metabolic products are expressed normalized to 10^6 cells. n represents the number of separate experiments performed under each set of conditions, with each experiment utilizing a distinct hepatocyte preparation. All values reported are means $\pm$ S.E.M. Statistical significance was determined by Student's *t* test, with $P < 0.05$ considered significant.

Reagents

All chemicals used were of reagent grade. [1-¹⁴C]-Butyrate and [1-¹⁴C]octanoate were obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). [1-¹⁴C]Pentanoate was purchased from ICN Radiochemicals (Costa Mesa, CA, U.S.A.). L-Carnitine was a gift from Sigma Tau (Rome, Italy). Collagenase (type II) was purchased from Cooper Biochemicals (Freehold, NJ, U.S.A.). Phosphotransacetylase was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.), and

malate dehydrogenase and citrate synthase were purchased from Boehringer Mannheim (Indianapolis, IN, U.S.A.).

RESULTS

Initial studies examined the rate of ketone-body formation (the sum of β -hydroxybutyrate and acetoacetate) from short- and medium-chain-length fatty acids in isolated rat hepatocytes. When 0.8 mM-butyrate, -hexanoate or -octanoate was the substrate, rates of ketogenesis were not different and averaged approx. 135 nmol of ketone bodies/10 min per 10^6 hepatocytes (Table 1). In contrast, ketone-body synthesis from pentanoate was only 19 ± 3 nmol/10 min per 10^6 hepatocytes ($n = 7$). As the substrate carbon-chain length was increased from five to seven (heptanoate) to nine (nonanoate), the rate of ketone-body formation was significantly increased (Table 1).

The slow rate of ketogenesis from odd-chain-length fatty acids suggested that generation of propionyl-CoA inhibited β -oxidation or generation of acetoacetate from acetyl-CoA. To examine this possibility, the effects of 10.0 mM-propionate on ketogenesis from butyrate, hexanoate and octanoate were studied. Propionate inhibited ketone body formation from butyrate by 81 % (Table 1). In contrast, ketogenesis from hexanoate was inhibited by 53 % and that from octanoate by only 18 % in the presence of propionate. The higher rates of ketone-body formation from odd-chain-length fatty acids with increasing chain length, and the decreased inhibition by propionate of ketogenesis from even-chain-length fatty acids as the chain length increased, suggested a site of inhibition in the metabolism of fatty acids that was relatively specific for short chain lengths.

If propionyl-CoA is responsible for the observed inhibition of ketogenesis, carnitine might increase the rates of ketone-body formation under these conditions, secondarily to production of propionylcarnitine. As would be expected, carnitine (10.0 mM) had no effect on the rates of ketone-body formation from butyrate, hexanoate or octanoate (Table 1). However, carnitine increased the rate of ketone-body formation from pentanoate and heptanoate by 53 % and 28 % respectively. Carnitine had no effect on ketogenesis from nonanoate. In contrast with the lack of effect of carnitine

on ketogenesis from butyrate alone, carnitine increased the rate of ketone-body formation from butyrate in the presence of propionate by 26 %. Ketone-body production from hexanoate in the presence of propionate was increased by 19 % by 10.0 mM-carnitine (Table 1). Thus, as in other systems [7,11], carnitine could partially reverse the inhibitory effects of propionate on cellular metabolism.

The inhibition of ketogenesis from butyrate by propionate was concentration-dependent (Table 2). Concentrations of propionate as low as 0.4 mM significantly inhibited ketone-body production from butyrate. Carnitine also increased the rate of ketogenesis from butyrate in the presence of 4.0 mM-propionate (42 ± 5 versus 51 ± 6 nmol of ketone bodies/10 min per 10^6 cells in the absence and presence of carnitine respectively; $P < 0.05$, $n = 8$). In addition to decreasing total ketone-body synthesis, propionate also increased the ratio of acetoacetate to β -hydroxybutyrate generated (Table 2).

Although ketone bodies are the major product of hepatic fatty acid oxidation, CO_2 is also formed. CO_2 formation from [$1\text{-}^{14}\text{C}$]butyrate represented approx. 14 % of total butyrate oxidized (Table 3; cf. Table 1). This rate of CO_2 generation was decreased by 58 % in the presence of 10.0 mM-propionate (Table 3). Carnitine added to incubations containing butyrate alone decreased CO_2 production from butyrate. However, under conditions in which CO_2 formation from butyrate was inhibited by propionate, 10.0 mM-carnitine increased CO_2 formation by 19 %. α -Oxobutyrate, which is metabolized to propionyl-CoA [28,29], also inhibited CO_2 formation from butyrate (22.6 ± 5.8 versus 10.8 ± 3.1 nmol of CO_2 /10 min per 10^6 hepatocytes in the absence and presence of 10.0 mM- α -oxobutyrate respectively, $P < 0.05$, $n = 6$). The rate of CO_2 production from [$1\text{-}^{14}\text{C}$]pentanoate was lower than that with butyrate as substrate, and was not affected by addition of carnitine. The rate of CO_2 formation from [$1\text{-}^{14}\text{C}$]octanoate was low and unaffected by carnitine, but was increased by addition of 10.0 mM-propionate.

The above studies were all performed in hepatocytes isolated from fed rats. Previous studies have demonstrated that propionate inhibition of palmitate oxidation was decreased in hepatocytes isolated from fasted rats as compared with cells from fed animals [7]. In hepatocytes isolated from 48 h-fasted rats, ketone-body

Table 1. Interaction of carnitine and propionate on ketone-body formation from short- and medium-chain-length fatty acids

Incubations containing $2.6(\pm 0.2) \times 10^6$ hepatocytes/ml ($n = 22$) were conducted as described in the text. All substrates were added to a final concentration of 0.8 mM. Values are means $\pm$ S.E.M. (n is shown in parentheses) for the net total ketone-body synthesis from time 0 to 10 min, and are given in nmol of ketone bodies generated/10 min per 10^6 hepatocytes: * $P < 0.05$ versus control; † $P < 0.05$ versus substrate plus propionate.

Substrate	Additions...	Ketone-body formation (nmol/10 min per 10^6 hepatocytes)			
		Control	Carnitine (10 mM)	Propionate (10 mM)	Carnitine + propionate
Butyrate		141 ± 10 (21)	140 ± 15 (16)	27 ± 4 (16)*	34 ± 5 (12)*†
Pentanoate		19 ± 3 (7)	29 ± 4 (5)*	—	—
Hexanoate		135 ± 19 (8)	126 ± 6 (4)	64 ± 14 (8)*	76 ± 21 (6)*†
Heptanoate		39 ± 6 (7)	50 ± 4 (7)*	—	—
Octanoate		129 ± 13 (5)	125 ± 16 (4)	106 ± 17 (5)	—
Nonanoate		77 ± 11 (6)	76 ± 9 (4)	—	—

Table 2. Propionate inhibition of ketone-body formation from butyrate

Incubations containing $2.8(\pm 0.3) \times 10^6$ hepatocytes/ml ($n = 16$) and 0.8 mM-butyrate were conducted as described in the text, and samples were removed at times 0 and 10 min for determination of acetoacetate and β -hydroxybutyrate concentrations. These values were used to calculate the net production rates of acetoacetate (AcAc), β -hydroxybutyrate (β -OH) and total ketone bodies (the sum of acetoacetate and β -hydroxybutyrate). The ratio of the rate of acetoacetate production to the rate of β -hydroxybutyrate production was also calculated for individual experiments, and the mean ratio was calculated. Values are nmol/10 min per 10^6 hepatocytes, and are means $\pm$ S.E.M.; * $P < 0.05$ versus zero propionate.

Propionate (mM)	<i>n</i>	Ketone-body formation (nmol/10 min per 10^6 hepatocytes)			
		AcAc	β -OH	AcAc/ β -OH	Total
0	16	39 $\pm$ 5	95 $\pm$ 8	0.42 $\pm$ 0.04	134 $\pm$ 13
0.1	5	38 $\pm$ 4	70 $\pm$ 12	0.63 $\pm$ 0.11	108 $\pm$ 14
0.4	4	29 $\pm$ 3	70 $\pm$ 17	0.71 $\pm$ 0.35*	99 $\pm$ 18*
1.0	4	28 $\pm$ 9	42 $\pm$ 13	0.67 $\pm$ 0.09*	70 $\pm$ 26*
4.0	11	22 $\pm$ 5	26 $\pm$ 5	0.91 $\pm$ 0.13*	48 $\pm$ 33*
10.0	16	10 $\pm$ 2	16 $\pm$ 3	1.14 $\pm$ 0.37*	26 $\pm$ 4*

Table 3. Production of $^{14}\text{CO}_2$ from 1- ^{14}C -labelled fatty acids

Incubations containing $2.3(\pm 0.3) \times 10^6$ hepatocytes were conducted as described in the text. At zero time, 1- ^{14}C -labelled substrates were added (final fatty acid concn. 0.8 mM, sp. radioactivity approx. 50 d.p.m./nmol). Reactions were stopped at times 0 and 10 min, and net $^{14}\text{CO}_2$ production was determined. Values are nmol of CO_2 /10 min per 10^6 hepatocytes, and are means $\pm$ S.E.M. (n is shown in parentheses): * $P < 0.05$ versus control; † $P < 0.05$ versus substrate plus propionate.

Substrate	Addition ...	$^{14}\text{CO}_2$ produced (nmol/10 min per 10^6 hepatocytes)			
		Control	Carnitine (10 mM)	Propionate (10 mM)	Carnitine + propionate
Butyrate		22.6 $\pm$ 5.8 (6)	13.3 $\pm$ 2.3 (4)*	9.5 $\pm$ 1.5 (5)*	11.3 $\pm$ 1.7 (4)*†
Pentanoate		13.6 $\pm$ 1.6 (6)	12.6 $\pm$ 3.4 (5)	—	—
Octanoate		3.4 $\pm$ 0.7 (5)	5.0 $\pm$ 2.5 (3)	5.7 $\pm$ 1.5 (5)	—

production from 10 mM-butyrate was 73 ± 7 nmol/10 min per 10^6 cells. This rate was decreased to 17 ± 2 nmol/10 min per 10^6 cells by the addition of 10 mM-propionate. Thus, propionate's inhibition of ketogenesis from butyrate was of a similar magnitude in hepatocytes from fasted rats (77%) to that with cells from fed rats (81%).

Fast-atom-bombardment mass spectroscopy was used to characterize the acylcarnitines formed during the metabolism of butyrate and propionate (Table 4). During a 60 min incubation with butyrate, significant amounts of acetylcarnitine were generated. Addition of 10.0 mM-propionate abolished the increase in acetylcarnitine, and a large increase in propionylcarnitine was observed. When pentanoate was the substrate, production of acetylcarnitine and propionylcarnitine was seen. Under none of the conditions studied were significant amounts of butyrylcarnitine or pentanoylcarnitine generated. These profiles of acylcarnitines were confirmed by using a h.p.l.c. resolution technique [30,31] coupled with radioenzymic determination of carnitine, and by employing the same resolution methodology with samples from incubations with radiolabelled fatty acid substrates (results not shown).

The above studies suggested that formation of propionyl-CoA was associated with inhibition of ketogenesis at the level of acetyl-CoA generation, and that

the effect of carnitine to increase ketone-body synthesis was mediated through an interaction with the CoA pool. To examine this possibility further, the status of the hepatocyte CoA pool during metabolism of short- and medium-chain-length fatty acids was studied. After 10 min of incubation with butyrate, hepatocyte CoA and acetyl-CoA contents were 1.32 and 0.53 nmol/ 10^6 cells respectively (Table 5). Addition of 10 mM-propionate decreased CoA by 54% and acetyl-CoA by 53%. In the presence of propionate, propionyl-CoA increased from undetectable in the absence of propionate to 0.28 nmol/ 10^6 cells. Addition of 10 mM-carnitine in the presence of butyrate and propionate increased the sum of the acetyl-CoA and CoA contents by 24%, as the propionyl-CoA content was decreased by 50%. Under no conditions were measurable amounts of butyryl-CoA seen. During oxidation of octanoate, addition of propionate also decreased CoA and acetyl-CoA, but not to the very low amounts seen when propionate was added during the oxidation of butyrate. Acid-insoluble CoA (long-chain acyl-CoA) contents were approx. 0.16 nmol/ 10^6 hepatocytes, and total acid-soluble CoA contents (the sum of CoA and all short-chain acyl-CoAs) were approx. 2.3 nmol/ 10^6 cells. The amounts of acid-insoluble CoA and total acid-soluble CoA were not changed during incubation with any of the substrates or inhibitors used in these studies.

Table 4. Acylcarnitine production during fatty acid metabolism

Incubations containing $10.2(\pm 1.6) \times 10^6$ hepatocytes/ml were conducted as described in the text with the fatty acids indicated (final concn. 0.8 mM) added at zero time. All incubations contained 10.0 mM-carnitine. Where indicated, the propionate concentration was 10.0 mM. At times 0 and 60 min, samples were removed and analysed for acylcarnitines by fast-atom-bombardment mass spectroscopy. Octanoylcarnitine was added as an external standard. Data are expressed as the ratio of the signal intensity at the m/z of interest (acetylcarnitine = 204, propionylcarnitine = 218, butyrylcarnitine = 232, pentanoylcarnitine = 246) normalized to the octanoylcarnitine signal (m/z = 288). The amount of octanoylcarnitine added was equivalent to approx. 400 nmol of acylcarnitine/ml of incubation. Values are means $\pm$ S.E.M.

Acylcarnitine	Time ... Fatty acid ... <i>n</i> ...	Acylcarnitine production			
		0 Butyrate 5	60 min Butyrate 5	60 min Butyrate + propionate 5	60 min Pentanoate 6
Acetyl		0.085 $\pm$ 0.014	0.152 $\pm$ 0.016	0.084 $\pm$ 0.006	0.135 $\pm$ 0.006
Propionyl		0.033 $\pm$ 0.007	0.030 $\pm$ 0.002	0.255 $\pm$ 0.022	0.049 $\pm$ 0.006
Butyryl		0.027 $\pm$ 0.006	0.030 $\pm$ 0.003	0.030 $\pm$ 0.003	0.028 $\pm$ 0.005
Pentanoyl		0.056 $\pm$ 0.009	0.048 $\pm$ 0.004	0.053 $\pm$ 0.010	0.063 $\pm$ 0.008

Table 5. Hepatocyte CoA pool during oxidation of short- and medium-chain-length fatty acids

Hepatocytes were isolated from fed rats, and incubations containing $11.3(\pm 1.1) \times 10^6$ hepatocytes/ml conducted as described in the text. After 10 min of incubation, samples were removed for determination of acetyl-CoA, the sum of acetyl-CoA and CoA, or propionyl-CoA as described in the text. CoA was determined as the difference in mean values for acetyl-CoA and the sum of acetyl-CoA and CoA. Carnitine and propionate were present at 10 mM where indicated, and all substrates were present at 0.8 mM. Values are nmol/ 10^6 cells, and are means $\pm$ S.E.M., with *n* shown in parentheses: **P* < 0.05 versus same substrate in the absence of propionate; †*P* < 0.05 versus identical conditions in the absence of carnitine. N.D., not determined.

Substrate	Additions	CoA + acetyl-CoA	Acetyl-CoA	CoA	Propionyl-CoA
None	None	1.85 $\pm$ 0.17 (6)	0.50 $\pm$ 0.07 (9)	1.35	< 0.02 (5)
Butyrate	None	1.85 $\pm$ 0.23 (6)	0.53 $\pm$ 0.08 (9)	1.32	< 0.02 (7)
Butyrate	Propionate	0.86 $\pm$ 0.16 (6)*	0.25 $\pm$ 0.05 (9)*	0.61	0.28 $\pm$ 0.07 (7)*
Butyrate	Propionate + carnitine	1.07 $\pm$ 0.09 (6)†	0.31 $\pm$ 0.05 (9)	0.76	0.14 $\pm$ 0.03 (7)†
Pentanoate	None	0.96 $\pm$ 0.12 (6)	0.28 $\pm$ 0.06 (8)	0.68	0.08 $\pm$ 0.03 (6)
Pentanoate	Carnitine	1.21 $\pm$ 0.09 (6)†	0.52 $\pm$ 0.14 (8)†	0.69	0.03 $\pm$ 0.02 (6)†
Octanoate	None	2.10 $\pm$ 0.32 (4)	0.62 $\pm$ 0.11 (7)	1.48	N.D.
Octanoate	Propionate	1.09 $\pm$ 0.36 (4)*	0.38 $\pm$ 0.06 (7)*	0.71	N.D.

DISCUSSION

The accumulation of propionate, or its activated form propionyl-CoA, is associated with the disruption of normal metabolic function in a variety of systems secondary to inhibition of a variety of metabolic pathways and important enzymes [3–7,9,11,32,33]. The present studies demonstrate that accumulation of propionyl-CoA, whether generated from exogenous propionate or endogenously from metabolic precursors, is associated with inhibition of ketogenesis from short and medium chain length fatty acids in hepatocytes. Further, the inhibition by propionyl-CoA could be modulated by carnitine, consistent with the observed effects of carnitine on the cellular CoA pool.

The ability of propionate to inhibit ketogenesis has been observed previously in isolated hepatocytes [7,20] and *in vivo* [19]. In the present studies, inhibition of ketogenesis by propionate was strongly dependent on the

chain length of the substrate fatty acid used. Propionate strongly inhibited ketogenesis from butyrate, inhibited ketogenesis from hexanoate weakly and had almost no effect on ketogenesis from octanoate. A similar pattern was seen in the metabolism of odd-chain-length fatty acids, which generate propionyl-CoA during their oxidation. Pentanoate was oxidized very slowly, whereas rates of nonanoate oxidation were similar to those observed with octanoate as substrate. This same chain-length dependence for acetoacetate formation from odd-chain-length fatty acids was reported by Witter *et al.* [34], using isolated mitochondria. Propionate decreased $^{14}\text{CO}_2$ production from [1- ^{14}C]butyrate, ruling out a shunting of acetyl-CoA away from acetoacetate formation towards citrate generation. Thus, although no specific mechanism can be defined, these observations are consistent with inhibition by propionyl-CoA of a step in fatty acid oxidation specific for short-chain-length fatty acids as compared with longer-chain-length fatty acids. Further,

the inhibition of butyrate metabolism by propionate must result from a mechanism distinct from that responsible for propionate's inhibition of palmitate oxidation [7,11].

The dramatic effects of propionate on the cellular CoA pool to decrease acetyl-CoA and CoA (Table 5) are similar to those reported by other investigators [8,24,35,36]. The similarity in the CoA pool during oxidation of pentanoate to that during the oxidation of butyrate in the presence of propionate emphasizes the key role of propionyl-CoA formation on the perturbations of the cellular CoA pool observed. Carnitine, through the formation of propionylcarnitine from propionyl-CoA, has been suggested to improve metabolic function under conditions of inhibition secondary to propionyl-CoA accumulation [7,12-14,37]. Carnitine demonstrated similar effects in the present studies, increasing ketogenesis from short- and medium-chain-length fatty acids only under those conditions where ketogenesis was markedly inhibited secondarily to exogenous propionate or when propionyl-CoA was generated during odd-chain-length fatty acid oxidation. Importantly, the current studies demonstrate that incubation in the presence of high concentrations of carnitine was associated with a decrease in propionyl-CoA and an increase in CoA and acetyl-CoA. High concentrations of carnitine were used to optimize propionylcarnitine formation [31], and to prevent the carnitine acyltransferase system from reaching equilibrium, which would minimize changes in the CoA pool [38]. Consistent with these concepts, treatment of children with organic acidurias appears to require frequent administration of large amounts of carnitine [17].

Methylmalonyl-CoA is known to accumulate under conditions of increased propionyl-CoA generation [8,24,35,36], and to be a potential inhibitor of metabolic processes [39,40]. However, no production of methylmalonylcarnitine was observed in the present or previous studies [7,31], suggesting that, like other branched-chain acyl-CoAs, it is a poor substrate for the carnitine acyltransferase system [41]. This may explain why quantitatively the beneficial effects of carnitine are relatively small, as methylmalonyl-CoA may accumulate to amounts greater than propionyl-CoA [24] (also shown in Table 5 as the difference between the fall in the amount of CoA plus acetyl-CoA and that of propionyl-CoA).

The present studies provide further evidence of the disruption of normal hepatic metabolism that may result secondarily to propionyl-CoA accumulation. Carnitine can partially reverse the detrimental effects resulting from a build-up of propionyl-CoA. The action of carnitine appears to result from propionylcarnitine generation and the consequent increase in CoA and decrease in propionyl-CoA. These results support the potential efficacy of carnitine as a scavenger for accumulating, potentially toxic, acyl-CoA derivatives [37].

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REFERENCES

1. Fritz, I. B. & Yue, K. T. N. (1963) *J. Lipid Res.* **4**, 279-288
2. Bremer, J. (1983) *Physiol. Rev.* **63**, 1420-1480
3. Coude, F. X., Sweetman, L. & Nyhan, W. L. (1979) *J. Clin. Invest.* **64**, 1544-1551
4. Stumpf, D. A., McAfee, J., Parks, J. K. & Eguren, L. (1980) *Pediatr. Res.* **35**, 1041-1045
5. Gregersen, N. (1981) *Biochem. Med.* **26**, 20-27
6. Davies, S. E. C., Frenkiel, T., Chalmers, R. A. & Iles, R. A. (1987) *Biochem. Soc. Trans.* **15**, 150-152
7. Brass, E. P., Fennessey, P. V. & Miller, L. V. (1986) *Biochem. J.* **236**, 131-136
8. Patel, T. B., DeBuysere, M. S. & Olson, M. S. (1983) *Arch. Biochem. Biophys.* **220**, 405-414
9. Gregersen, N. (1979) *Pediatr. Res.* **13**, 1227-1330
10. Chalmers, R. A. & Lawson, A. M. (1982) *Organic Acids in Man: The Analytical Chemistry, Biochemistry and Diagnosis of the Organic Acidurias*, Chapman and Hall, London
11. Brass, E. P. (1986) *Biochim. Biophys. Acta* **888**, 18-24
12. Bohles, H. & Lehnert, W. (1984) *Eur. J. Pediatr.* **143**, 61-63
13. Roe, C. R., Millington, D. S., Maltby, D. A., Bohan, T. P. & Hoppel, C. L. (1984) *J. Clin. Invest.* **73**, 1785-1788
14. Seccombe, D. W., Snyder, F. & Parsons, H. G. (1982) *Lancet* **ii**, 1401
15. Roe, C. R., Hoppel, C. L., Stacey, T. E., Chalmers, R. A., Tracey, B. M. & Millington, D. S. (1983) *Arch. Dis. Child.* **58**, 916-920
16. Roe, C. R., Millington, D. S., Maltby, D. A., Kahler, S. G. & Bohan, T. P. (1984) *J. Clin. Invest.* **74**, 2290-2295
17. Chalmers, R. A., Stacey, T. E., Tracey, B. M., DeSousa, C., Roe, C. R., Millington, D. S. & Hoppel, C. L. (1984) *J. Inherited Metab. Dis.* **7**, Suppl. 2, 109-110
18. DeSousa, C., Chalmers, R. A., Stacey, T. E., Tracey, B. M., Weaver, C. M. & Bradley, D. (1986) *Eur. J. Pediatr.* **144**, 451-456
19. Williams, D. L., Spray, G. H., Hems, R. & Williamson, D. H. (1971) *Biochem. J.* **124**, 501-507
20. Bates, A. J. & Dickson, A. J. (1984) *Comp. Biochem. Physiol.* **78B**, 437-442
21. Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506-520
22. Brass, E. P., Garrity, M. J. & Robertson, R. P. (1984) *FEBS Lett.* **169**, 293-296
23. Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., pp. 1988-1993, Academic Press, New York
24. Corkey, B. E., Brandt, M., Williams, R. J. & Williamson, J. R. (1981) *Anal. Biochem.* **118**, 30-41
25. Allred, J. B. & Guy, D. G. (1969) *Anal. Biochem.* **29**, 293-297
26. Olsen, C. (1971) *Clin. Chim. Acta* **33**, 293-300
27. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) *J. Biol. Chem.* **177**, 751-766
28. Ciman, M. & Siliprandi, N. (1968) *Biochim. Biophys. Acta* **162**, 164-169
29. Bremer, J. (1969) *Eur. J. Biochem.* **8**, 535-540
30. Hoppel, C. L., Brass, E. P., Gibbons, A. P. & Turkaly, J. S. (1986) *Anal. Biochem.* **156**, 111-117
31. Brass, E. P. & Beyerinck, R. A. (1987) *Metab. Clin. Exp.* **36**, 781-787
32. Chan, T. M. & Freedland, R. A. (1972) *Biochem. J.* **127**, 539-543
33. Glasgow, A. M. & Chase, H. P. (1976) *Pediatr. Res.* **10**, 683-686
34. Witter, R. F., Cottune, M. A. & Stotz, E. (1954) *J. Biol. Chem.* **207**, 671-678

35. LaPointe, D. S. & Olson, M. S. (1985) *Arch. Biochem. Biophys.* **242**, 417–429
36. Martin-Requero, A., Corkey, B. E., Walajtys-Rude, E., Parrilla, R. L. & Williamson, J. R. (1983) *J. Biol. Chem.* **258**, 3673–3681
37. Chalmers, R. A., Roe, C. R., Tracey, B. M., Stacey, T. E., Hoppel, C. L. & Millington, D. S. (1983) *Biochem. Soc. Trans.* **11**, 724–725
38. Brass, E. P. & Hoppel, C. L. (1980) *Biochem. J.* **190**, 495–504
39. Scrutton, M. C. & Utter, M. F. (1967) *J. Biol. Chem.* **242**, 1723–1735
40. Arinze, J. J., Waters, D. & Donaldson, M. K. (1979) *Biochem. J.* **184**, 717–719
41. Solberg, H. E. & Bremer, J. (1970) *Biochim. Biophys. Acta* **222**, 372–380

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