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Effect of hydroxycobalamin[c-lactam] on propionate and carnitine metabolism in the rat

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The administration *in vivo* of the cobalamin analogue hydroxycobalamin[c-lactam] inhibits hepatic L-methylmalonyl-CoA mutase activity. The current studies characterize *in vivo* and *in vitro* the hydroxycobalamin[c-lactam]-treated rat as a model of disordered propionate and methylmalonic acid metabolism. Treatment of rats with hydroxycobalamin[c-lactam] (2 μg/h by osmotic minipump) increased urinary methylmalonic acid excretion from 0.55 μmol/day to 390 μmol/day after 2 weeks. Hydroxycobalamin[c-lactam] treatment was associated with increased urinary propionylcarnitine excretion and increased short-chain acylcarnitine concentrations in plasma and liver. Hepatocytes isolated from cobalamin-analogue-treated rats metabolized propionate (1.0 mM) to CO_2 and glucose at rates which were only 18% and 1% respectively of those observed in hepatocytes from control (saline-treated) rats. In contrast, rates of pyruvate and palmitate oxidation were higher than control in hepatocytes from the hydroxycobalamin[c-lactam]-treated rats. In hepatocytes from hydroxycobalamin[c-lactam]-treated rats, propionylcarnitine was the dominant product generated from propionate when carnitine (10 mM) was present. The addition of carnitine thus resulted in a 4-fold increase in total propionate utilization under these conditions. Hepatocytes from hydroxycobalamin[c-lactam]-treated rats were more sensitive than control hepatocytes to inhibition of palmitate oxidation by propionate. This inhibition of palmitate oxidation was partially reversed by addition of carnitine. Thus hydroxycobalamin[c-lactam] treatment *in vivo* rapidly causes a severe defect in propionate metabolism. The consequences of this metabolic defect *in vivo* and *in vitro* are those predicted on the basis of propionyl-CoA and methylmalonyl-CoA accumulation. The cobalamin-analogue-treated rat provides a useful model for studying metabolism under conditions of a metabolic defect causing acyl-CoA accretion.

INTRODUCTION

Cobalamin (vitamin B_{12}) is a required cofactor for the enzymes L-methylmalonyl-CoA mutase and methionine synthetase [1]. Cobalamin deficiency is of interest as a clinical disorder [2,3], in understanding the regulation of cobalamin metabolism [3] and as a model for disorders of intermediary metabolism [4]. Dietary cobalamin deficiency is difficult to produce in animals, requires long periods on special diets and yields a heterogeneous metabolic defect [4,5]. The administration *in vivo* of cobalamin analogues, including hydroxycobalamin[c-lactam], has been shown to cause decreased hepatic cobalamin levels, decreased hepatic L-methylmalonyl-CoA mutase activity and methylmalonic aciduria in the rat [6].

The decreased L-methylmalonyl-CoA mutase activity associated with cobalamin deficiency has several important metabolic consequences. Propionyl-CoA and methylmalonyl-CoA levels are increased in liver from vitamin B_{12}-deficient rats [7]. Propionate utilization is decreased in vitamin B_{12}-deficient rats [8,9], and methylmalonic acid concentrations are dramatically increased in plasma and urine [4,10]. The high tissue propionyl-CoA content results in increased propionylcarnitine content, as the propionyl group is reversibly transferred from the thioester to form the corresponding acylcarnitine [4,11]. Additionally, the accumulation of propionyl-CoA and methylmalonyl-CoA in cells alters the regulation of a number of important metabolic pathways [12–15]. Thus the metabolic consequences of cobalamin deficiency are important in understanding the potential pathophysiology of vitamin B_{12} deficiency, and in studies of metabolic regulation under conditions of acyl-CoA accretion.

The current studies were undertaken to characterize the effect of hydroxycobalamin[c-lactam] treatment on propionate and carnitine metabolism in the rat. The results demonstrate that treatment causes a severe impairment in propionate metabolism and alterations in carnitine metabolism consistent with severe functional vitamin B_{12} deficiency.

MATERIALS AND METHODS

Animals and hepatocyte isolation

Male Fischer 344 rats were used in all studies. Rats were housed in individual wire-bottomed cages with access to water and rat chow (Agway, Longmont, CO, U.S.A.) *ad libitum*. After a 1 week acclimation period, an osmotic minipump (Alza Corporation, Palo Alto, CA, U.S.A.; model 2002) containing either saline (control) or hydroxycobalamin[c-lactam] (treated) was implanted

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subcutaneously in each animal. Hydroxycobalamin[c-lactam] was delivered at a rate of 2 μg/h. Each animal received a second minipump (contents identical in each animal to their initial minipump) 21 days after initial implantation. The rats weighed 220 ± 5 g (mean $\pm$ S.E.M., $n = 17$) at the time the first pumps were implanted.

At the times indicated, 24 h urine collections were made into 25 ml Erlenmeyer flasks containing 10 μl of 1.2 M-HCl which were kept on dry ice throughout the 24 h collection. Plasma, liver, skeletal muscle (hind leg) and heart samples were obtained from selected animals following decapitation. Tissue samples were collected by rapid freezing *in situ* in aluminium blocks cooled in dry ice/acetone. Samples were stored at -76 °C until assays were performed.

Hepatocytes were prepared by a modification of the collagenase perfusion technique of Berry & Friend [16] as detailed previously [17]. Rats used for hepatocyte isolation were in the fed state and the preparations were initiated at 07:00 h. Rats weighed 300 ± 10 g at the time of cell isolation, and the hepatocyte preparations were 91 ± 1% viable based on Trypan Blue exclusion, contained 3.02 ± 0.17 mg of protein/10^6 hepatocytes and had a wet weight of 14.2 ± 1.04 mg/10^6 hepatocytes (all means $\pm$ S.E.M., $n = 6$). There were no differences in any of these parameters when comparing hepatocytes from control and hydroxycobalamin[c-lactam]-treated animals.

Assays

Carnitine was quantified using the radioenzymic assay of Cederblad & Lindstedt [18], modified as detailed previously [19]. Carnitine concentrations in the stock carnitine standard were verified using the method of Marquis & Fritz [20]. Plasma and tissue samples were prepared for analysis in 3% $HClO_4$. The $HClO_4$-soluble fraction was assayed directly for carnitine, and after alkaline hydrolysis for measurement of total acid-soluble carnitine. Total acid-soluble carnitine is the sum of carnitine and short-chain acylcarnitines (acylcarnitines with acyl groups of less than ten carbon atoms in length). The sample short-chain acylcarnitine content was calculated as the total acid-soluble carnitine content minus the carnitine content. The $HClO_4$-insoluble fraction was hydrolysed with KOH at 65 °C for 1 h, neutralized and assayed for carnitine as a measure of long-chain acylcarnitine content (acylcarnitines with acyl groups of ten or more carbon atoms). Total carnitine refers to the sum of carnitine, short-chain acylcarnitines and long-chain acylcarnitines. Urine samples were assayed for carnitine and short-chain acylcarnitines as above, except that the $HClO_4$ fractionation was omitted.

Relative urinary propionylcarnitine concentrations were estimated using fast-atom-bombardment mass spectrometry as described previously [15]. Isobutyrylcarnitine was added as an external standard to each sample after partial purification of the propionylcarnitine on Dowex 1×8, Cl^- form. The propionylcarnitine content was characterized as the signal intensity at an m/z of 218 (propionylcarnitine) divided by the signal intensity at an m/z of 232 (isobutyrylcarnitine external standard), normalized to creatinine content (divided by mg of creatinine/ml).

Methylmalonic acid concentrations in urine were determined using the gas chromatography/mass spectrometry assay detailed previously [21]. Hepatocyte protein content was determined using the biuret method [22].

Hepatocyte metabolism

Metabolic studies using isolated hepatocytes were conducted in 25 ml Erlenmeyer flasks equipped with sidearms and centrewells. Incubation procedures are detailed elsewhere [15,23,24]. Briefly, incubations contained hepatocytes [$(2.86 \pm 0.23) \times 10^6$ cells/ml, mean $\pm$ S.E.M., $n = 6$], 134 mM-NaCl, 4.2 mM-KCl, 2.4 mM-Na_2HPO_4, 0.72 mM-$MgSO_4$, 0.9 mM-$CaCl_2$, 0.8 mM-Tris(hydroxymethyl)aminoethane, 4 mM-glucose and other compounds as indicated in individual experiments. Total incubation volume was 2.5 ml, and incubations were conducted at 37 °C under O_2/CO_2 (19:1). Following a 30 min preincubation, substrates were added at time zero. Substrate specific activities were 90 d.p.m./nmol for 1.0 mM-[1-^{14}C]propionate, 30 d.p.m./nmol for 10.0 mM-[1-^{14}C]propionate, 18 d.p.m./nmol for 10 mM-[1-^{14}C]pyruvate and 55 d.p.m./nmol for 0.8 mM-[1-^{14}C]palmitate.

Conversions of [1-^{14}C]propionate, [1-^{14}C]pyruvate and [1-^{14}C]palmitate to $^{14}CO_2$, [^{14}C]glucose and [^{14}C]acylcarnitines were quantified as detailed previously and validated [23,24]. For estimation of [^{14}C]glucose and [^{14}C]acylcarnitine content, aliquots were removed at 0 and 30 min and added to an equal volume of cold 6% (v/v) $HClO_4$. Neutralized samples were passed through a Dowex 1×8 Cl^- form mini-column either after alkaline hydrolysis (resulting eluent containing [^{14}C]glucose) or without alkaline hydrolysis (resulting eluent containing [^{14}C]glucose and [^{14}C]acylcarnitines). $^{14}CO_2$ was collected in flask centrewells containing 0.3 ml of ethanolamine/ethylene glycol monomethyl ether (1:2, v/v) after addition of $HClO_4$ to the incubation to yield a 3% $HClO_4$ concentration in the incubation. Formation of ^{14}C-labelled acid-soluble products from [1-^{14}C]palmitate was estimated as radioactivity in the $HClO_4$ supernatant from the incubations. In all cases, ^{14}C contained in time zero aliquots was subtracted to provide net ^{14}C-product formation. Calculations of substrate utilization or product formation were based on the substrate specific activity as detailed previously [23,24].

Reagents

[1-^{14}C]Propionate, [1-^{14}C]palmitate and [1-^{14}C]-pyruvate were purchased from New England Nuclear (Boston, MA, U.S.A.). [^{3}H]Acetyl-CoA used in the carnitine assay was purchased from ICN Biochemicals (Costa Mesa, CA, U.S.A.), and Dowex 1×8 Cl^- form (200–400 mesh) ion-exchange resin was from Bio-Rad, Inc. (Richmond, CA, U.S.A.). Carnitine acetyltransferase and acetyl-CoA were obtained from Sigma Chemicals (St Louis, MO, U.S.A.). Collagenase (Type II) used for the hepatocyte isolations was purchased from Worthington Biochemicals (Freehold, NJ, U.S.A.). CN-cobalamin[c-lactam] was synthesized, purified and analysed as described previously [25], and converted to the hydroxy form by reduction using acetic acid and zinc dust [26]. l-Carnitine and isobutyrylcarnitine were gifts from Sigma Tau (Rome, Italy). Other chemicals and reagents used were of reagent grade.

Statistics

All values presented are means $\pm$ S.E.M., with n referring to the number of individual animals or distinct hepatocyte

preparations used. Statistical significance was determined using Student's t test, with $P < 0.05$ considered to be significant.

RESULTS

Urinary methylmalonic acid excretion was quantified at weekly intervals to determine the time course of metabolic changes following hydroxycobalamin[c-lactam] administration (2 μg/h by osmotic minipump). Urinary methylmalonic acid excretion remained constant at 0.5–2.0 μmol/day during weeks 2, 4, 5 and 6 in control animals (osmotic minipumps containing saline). In contrast, methylmalonic acid excretion increased rapidly during administration of hydroxycobalamin[c-lactam] (Table 1). During week 2 of cobalamin-analogue treatment, methylmalonic acid excretion averaged 390 μmol/day ($n = 8$). Methylmalonic acid excretion fell to 7 μmol/day in the treated animals during week 4. The osmotic minipumps were changed early in week 4, as the pumps are developed by the manufacturer to deliver for a 2–3 week period. Following implantation of the second pump, urinary methylmalonic acid excretion again increased rapidly, reaching 150 μmol/day during week 5 and 220 μmol/day during week 6.

A group of control and hydroxycobalamin[c-lactam]-treated animals were killed (in the fed state) during week 6 to assess the plasma and tissue carnitine pools (Table 2). Hydroxycobalamin[c-lactam] treatment caused an increase in the plasma short-chain acylcarnitine concentration and a decrease in the plasma free carnitine concentration, whereas the total acid-soluble carnitine concentration was unchanged as compared with control animals. Short-chain acylcarnitines constituted 21% of the total plasma acid-soluble carnitine in the cobalamin-analogue-treated rats as compared with 8% in the saline-treated rats. A similar relative increase in short-chain acylcarnitines with cobalamin-analogue treatment occurred in the liver, with 71% of the total acid-soluble carnitine present as short-chain acylcarnitine as compared with 49% in controls. There were no changes in the acid-soluble carnitine pools in skeletal muscle or heart with hydroxycobalamin[c-lactam] treatment. Long-chain acylcarnitine content was decreased by 30% and 48% in liver and heart respectively by cobalamin-analogue administration. Hydroxycobalamin[c-lactam] treatment caused an increase in urinary propionylcarnitine excretion as assessed by fast-atom-bombardment mass spectrometry (Table 3), and tended to increase total urinary acylcarnitine excretion.

Hepatocytes were isolated from saline-treated and from hydroxycobalamin[c-lactam]-treated rats during weeks 5 and 6 of administration to permit measurements of metabolic activity *in vitro*. Rates of $^{14}CO_2$ and [^{14}C]glucose production from [1-^{14}C]propionate (1.0 mM)

Table 1. Urinary methylmalonic acid excretion in control and hydroxycobalamin[c-lactam]-treated rats

Urine was collected for 24 h from saline-treated and from hydroxycobalamin[c-lactam]-treated rats during weeks 1–6 of treatment. Urinary methylmalonic acid concentrations were determined and the daily methylmalonic acid excretion (μmol/day) was calculated in each case. Values are means ± S.E.M. with n in parentheses. N.D., not determined.

	Urinary methylmalonic acid (μmol/day)	
Week \ Treatment...	Saline	Hydroxy-cobalamin[c-lactam]
1	N.D.	0.86±0.20 (9)
2	0.55±0.14 (9)	390±260 (8)
3	N.D.	81±14 (8)
4	1.82±0.78 (9)	6.7±2.9 (7)
5	0.76±0.17 (8)	150±50 (6)
6	0.47±0.13 (7)	220±70 (6)

Table 2. Effect of hydroxycobalamin[c-lactam] treatment on tissue carnitine pools in the rat

Rats treated with saline (control) or hydroxycobalamin[c-lactam] (treated) were killed after 6 weeks of treatment. Plasma, liver, skeletal muscle and heart were collected and free carnitine, short-chain acylcarnitine, total acid-soluble carnitine (TAS; free carnitine + short-chain-acylcarnitine), long-chain acylcarnitine and total carnitine content were determined. SC/TAS refers to the ratio of the short-chain acylcarnitine content to the total acid-soluble acylcarnitine content. Values are given in μM (plasma) or nmol/g wet weight, and are means ± S.E.M. with $n = 6$ for control and $n = 4$ for treated rats. * Significantly different from control; $P < 0.05$.

	Free carnitine	Short-chain acylcarnitine	Total acid-soluble carnitine	SC/TAS	Long-chain acylcarnitine	Total carnitine
Plasma						
Control	71.8±1.5	6.5±2.0	78.4±3.0	0.080±0.022	6.82±0.31	85.3±3.3
Treated	63.0±1.7*	17.3±4.8*	80.4±5.4	0.207±0.048*	6.47±0.35	86.8±5.4
Liver						
Control	119±7	115±10	234±13	0.488±0.024	10±1	244±14
Treated	49±3*	121±6	170±3*	0.709±0.024*	7±1*	177±3*
Skeletal muscle						
Control	534±32	154±42	688±45	0.216±0.047	23±2	713±46
Treated	572±93	81±15	654±86	0.133±0.031	22±4	676±90
Heart						
Control	391±30	354±45	746±54	0.469±0.040	65±7	811±59
Treated	424±43	423±36	847±29	0.501±0.047	34±10*	881±38

Table 3. Effect of hydroxycobalamin[c-lactam] treatment on urinary carnitine excretion in the rat

Urine collections (24 h) were obtained from rats during the sixth week of treatment with saline (control) or hydroxycobalamin[c-lactam] (treated). Urinary contents of free carnitine and short-chain acylcarnitines were determined, and the ratio of the short-chain (SC) acylcarnitine excretion to the total carnitine excretion was calculated. Urine samples were analysed using fast-atom-bombardment mass spectroscopy (f.a.b.m.s.) to express the propionylcarnitine content (signal intensity $m/z = 218$) to an isobutyrylcarnitine external standard (signal intensity $m/z = 232$) normalized to creatinine content. Values are means ± S.E.M. ($n = 5$). * Significantly different from control; $P < 0.05$.

Animals	Free carnitine (nmol/day)	Short-chain acylcarnitine (nmol/day)	SC-acylcarnitine/ total carnitine	F.a.b.m.s. signal intensity (218/232)
Control	710±130	350±100	0.31±0.03	1.14±0.16
Treated	680±190	500±160	0.44±0.08	3.62±0.91*

Table 4. Effect of hydroxycobalamin[c-lactam] treatment *in vivo* on hepatocyte metabolism

Hepatocytes were isolated from rats during the fifth and sixth weeks of treatment with saline (control) or hydroxycobalamin[c-lactam] (treated). Metabolism of 1-^{14}C-substrates was studied, and values are nmol of substrate metabolized/30 min per 10^6 hepatocytes means ± S.E.M.; $n = 3$ for all determinations. * Significantly different from controls; $P < 0.05$. N/A, not applicable.

Substrate	CO_2 production	Glucose production	Acid-soluble product formation
1.0 mM-Propionate			
Control	70.5±12.1	30.3±1.8	N/A
Treated	12.7±1.9*	0.3±0.1*	N/A
10.0 mM-Propionate			
Control	69.9±9.4	29.3±1.2	N/A
Treated	15.9±0.8*	0.9±0.5*	N/A
10.0 mM-Pyruvate			
Control	225±8	34.7±3.1	N/A
Treated	327±20*	34.5±4.3	N/A
0.8 mM-Palmitate			
Control	5.9±0.5	N/A	27.3±2.2
Treated	11.2±1.6*	N/A	53.0±5.3*

in cells from cobalamin-analogue-treated rats were 82% and 99% respectively less than those observed in hepatocytes from saline-treated rats (Table 4). A similar impairment in propionate metabolism by hepatocytes from analogue-treated rats was observed with 10 mM substrate. In contrast, hepatocyte [^{14}C]glucose production from [1-^{14}C]pyruvate was unaffected by hydroxycobalamin[c-lactam] treatment of the rats, whereas rates of $^{14}CO_2$ generation from [1-^{14}C]pyruvate and [1-^{14}C]palmitate oxidation were increased by the cobalamin-analogue treatment.

Addition of carnitine (10 mM) to incubations containing hepatocytes from control rats increased propionylcarnitine production by 14- and 2-fold from 1.0 and 10.0 mM-propionate respectively, thus increasing total hepatocytes propionate utilization by 13–29% (Table 5). In the presence of 10 mM-carnitine, rates of propionylcarnitine production were similar using hepatocytes from control and hydroxycobalamin[c-lactam]-treated rats. Addition of carnitine increased total propionate utilization by 3.4–4.0-fold in cells from cobalamin-analogue-treated rats.

In hepatocytes from control rats, 1.0 mM-propionate inhibited [1-^{14}C]palmitate (0.8 mM) oxidation by 33%, and this inhibition was abolished by the addition of 10 mM-carnitine (Table 6). In contrast, 1.0 mM-propionate inhibited [1-^{14}C]palmitate oxidation by 83% in hepatocytes from hydroxycobalamin[c-lactam]-treated animals. In hepatocytes from hydroxycobalamin[c-lactam]-treated rats, carnitine (10 mM) increased palmitate oxidation 4-fold when propionate (1.0 mM) was present in the incubation. Thus hepatocytes from hydroxycobalamin[c-lactam]-treated rats were more sensitive to the inhibitory effects of propionate on palmitate metabolism, and carnitine was able to partially reverse this effect of propionate.

DISCUSSION

The interaction of carnitine with the cellular CoA pool, through the production of acylcarnitines, is potentially critical for maintaining normal cellular metabolism under conditions of impaired acyl-CoA utilization. An understanding of the dynamic effect of carnitine on metabolic regulation under these conditions of acyl-CoA build-up has been limited by the lack of experimental models that permit the interaction between the carnitine pool, the CoA pool and metabolic activity to be studied.

Table 5. Effect of carnitine on hepatocyte propionate utilization

Hepatocytes isolated from rats receiving saline (control) or hydroxycobalamin[c-lactam] (treated) were incubated with [1-^{14}C]propionate at the concentrations indicated in the absence (−CN) or presence (+CN) of 10 mM-carnitine. Propionylcarnitine production and total propionate utilization (sum of propionate converted to CO_2, glucose and propionylcarnitine) were determined. Values are means ± S.E.M. ($n = 3$) and are expressed as nmol of propionate/30 min per 10^6 cells.

	Propionylcarnitine production		Total propionate utilization	
	−CN	+CN	−CN	+CN
1.0 mM-Propionate				
Control	2.4±0.6	33.8±3.2	103±14	133±15
Treated	0.9±0.2	41.2±4.2	14±2	56±6
10.0 mM-Propionate				
Control	13.7±0.6	32.0±1.9	113±18	128±14
Treated	0.7±0.3	46.3±4.5	20±1	69±8

Table 6. Effect of propionate on palmitate oxidation

Hepatocytes were isolated from rats receiving saline (control) or hydroxycobalamin[c-lactam] (treated) and incubated with 0.8 mM-[1-^{14}C]palmitate. Formation of CO_2, acid-soluble (AS) products and total palmitate oxidation (total = CO_2 + AS products) was determined and is expressed as nmol of palmitate oxidized/30 min per 10^6 cells. When added, the propionate concentration was 1.0 mM, and the carnitine concentration was 10.0 mM. Values are means ± S.E.M., $n = 3$. *$P < 0.05$ versus control; †$P < 0.05$ versus identical conditions without propionate; ‡$P < 0.05$ versus identical conditions without carnitine.

Substrates	CO_2	AS products	Total
Palmitate			
Control	5.9±0.5	27.3±2.2	33.2±1.9
Treated	11.2±1.6*	53.0±5.3*	64.2±6.7*
Palmitate + carnitine			
Control	6.7±0.8	35.2±5.2	42.0±4.6
Treated	8.4±1.6	86.2±8.1*	95.9±9.5*
Palmitate + propionate			
Control	6.7±0.5	15.6±2.7†	22.3±2.3†
Treated	4.6±0.2	6.1±0.1*†	10.7±0.3*†
Palmitate + carnitine + propionate			
Control	8.4±1.0	33.0±3.6‡	41.3±4.2‡
Treated	12.7±1.9‡	30.6±1.4†‡	43.3±2.5†‡

The current studies demonstrate that administration of hydroxycobalamin[c-lactam] *in vivo* results in the rapid, severe development of metabolic abnormalities similar to dietary vitamin B_{12} (cobalamin) deficiency [4,11]. Using this model, the ability of carnitine to dramatically increase hepatic propionate utilization through propionylcarnitine production, despite decreased L-methylmalonyl-CoA mutase activity, has been demonstrated. The importance of this propionylcarnitine generation for carnitine homeostasis *in vivo* and for hepatocyte oxidative metabolism *in vitro* has also been delineated using the hydroxycobalamin[c-lactam]-treated rat model.

Urinary methylmalonic acid excretion was 700 times higher than control values after only 2 weeks of hydroxycobalamin[c-lactam] treatment. During week 4 of cobalamin-analogue treatment, urinary methylmalonic acid excretion had fallen to rates only 4-fold above control rates, corresponding to depletion of the first osmotic minipump and implantation of the second minipump. Profound methylmalonic aciduria rapidly redeveloped after the second minipump was implanted (Table 1), suggesting that the metabolic defect associated with hydroxycobalamin[c-lactam] treatment is rapidly reversed when the cobalamin analogue is no longer being delivered.

Dietary vitamin B_{12} deficiency results in decreased L-methylmalonyl-CoA activity [27], and as a consequence, in accumulation of propionyl-CoA and methylmalonyl-CoA [7], methylmalonic aciduria [10], decreased propionate metabolism [8,9] and increased acylcarnitine concentrations [4,11]. However, the development of dietary vitamin B_{12} deficiency requires 12–30 weeks of special feeding, diets and handling. Additionally, the magnitude of the metabolic defect with dietary vitamin B_{12} deficiency may be very heterogeneous [4]. However, the methylmalonic aciduria present after 2 weeks of hydroxycobalamin[c-lactam] treatment is as severe as in the most severely affected rats after 24 weeks of dietary vitamin B_{12} deficiency [4]. As in dietary vitamin B_{12} deficiency, hydroxycobalamin[c-lactam] treatment results in decreased methionine synthetase activity and a modest 4-fold increase in plasma homocysteine concentrations [28]. Altered methionine synthetase activity may also

influence intermediary metabolism in models of vitamin B_{12} deficiency, but does not preclude studies relevant to the impaired methylmalonyl-CoA mutase activity.

The status of the carnitine pool, particularly the distribution of total carnitine between short-chain acylcarnitines and free carnitine, provides a reflection of the metabolic state of the CoA pool [29,30]. Hydroxycobalamin[c-lactam] treatment increased urinary propionylcarnitine excretion and increased short-chain acylcarnitine content in plasma and liver (Tables 2 and 3). This increase in acylcarnitine is expected under conditions of acyl-CoA accumulation, and is identical to the change in carnitine metabolism with dietery vitamin B_{12} deficiency [4]. The long-chain acylcarnitine content of liver and heart decreased with hydroxycobalamin[c-lactam] treatment (Table 3). Accumulation of propionyl-CoA decreases fatty acid oxidation in liver [15,31] and fibroblasts [14]. Propionyl-CoA is an inhibitor of carnitine palmitoyltransferase [32], the enzyme responsible for long-chain acylcarnitine production, which is a potential rate-limiting step for fatty acid oxidation [33]. These effects of propionyl-CoA may explain the decreased long-chain acylcarnitine content with hydroxycobalamin[c-lactam] treatment. In liver, the lower long-chain acylcarnitine content may reflect the lower free carnitine content associated with cobalamin-analogue treatment (Table 3).

As with dietary vitamin B_{12} deficiency [34], the metabolic defect associated with hydroxycobalamin[c-lactam] treatment was demonstrable using hepatocytes isolated from treated rats. Total propionate utilization was decreased by 84% in hepatocytes from rats treated with hydroxycobalamin[c-lactam], whereas pyruvate and palmitate oxidation were increased. The effect of hydroxycobalamin[c-lactam] treatment on hepatocyte propionate metabolism could not be reproduced by incubating hepatocytes from control animals with 2 μg of hydroxycobalamin[c-lactam]/ml for 1.5 h (results not shown), further demonstrating that the action of the cobalamin analogue is not due to non-specific direct toxicity or metabolic actions.

Consistent with their decreased ability to metabolize propionate, hepatocytes isolated from hydroxycobalamin[c-lactam]-treated rats were more sensitive to metabolic inhibition by propionate than hepatocytes isolated from saline-treated rats (Table 6). In hepatocytes from cobalamin-analogue-treated rats, palmitate oxidation was inhibited by 83% in the presence of 1.0 mM-propionate, resulting in palmitate oxidation rates which were only 48% of those seen at identical substrate and inhibitor concentrations using cells from saline-treated rats. High concentrations of carnitine can increase propionate utilization via propionylcarnitine formation [24,35], acutely decreasing the propionyl-CoA concentration [36,37] and partially reversing the metabolic effects of propionyl-CoA accumulation [15,23,36]. Propionylcarnitine can represent a major product of hepatocyte propionate metabolism in vitamin B_{12} deficiency [34]. These concepts were also demonstrated using hepatocytes isolated from hydroxycobalamin-[c-lactam]-treated rats (Tables 4 and 5). These observations further demonstrate the importance of the carnitine–CoA pool interaction under conditions of acyl-CoA build-up.

Treatment of rats *in vivo* with hydroxycobalamin[c-lactam] results in the rapid development of a severe metabolic defect analogous to dietary vitamin B_{12} deficiency. This model can be used for studies *in vivo* and *in vitro* of metabolic regulation under conditions of decreased L-methylmalonyl-CoA mutase activity and acyl-CoA accretion. *In vitro*, carnitine partially normalized hepatocyte metabolism despite the underlying metabolic defect resulting from hydroxycobalamin[c-lactam] treatment. The hydroxycobalamin[c-lactam]-treated rat provides a useful model for studies of the consequences and treatment of metabolic disorders characterized by acyl-CoA accretion.

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