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Impact of short- and medium-chain organic acids, acylcarnitines, and acyl-CoAs on mitochondrial energy metabolism

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ABSTRACT

Accumulation of organic acids as well as their CoA and carnitine esters in tissues and body fluids is a common finding in organic acidurias, *beta*-oxidation defects, Reye syndrome, and Jamaican vomiting sickness. Pathomechanistic approaches for these disorders have been often focused on the effect of accumulating organic acids on mitochondrial energy metabolism, whereas little is known about the pathophysiologic role of short- and medium-chain acyl-CoAs and acylcarnitines. Therefore, we investigated the impact of short- and medium-chain organic acids, acylcarnitines, and acyl-CoAs on central components of mitochondrial energy metabolism, namely *alpha*-ketoglutarate dehydrogenase complex, pyruvate dehydrogenase complex, and single enzyme complexes I-V of respiratory chain. Although at varying degree, all acyl-CoAs had an inhibitory effect on pyruvate dehydrogenase complex and *alpha*-ketoglutarate dehydrogenase complex activity. Effect sizes were critically dependent on chain length and number of functional groups. Unexpectedly, octanoyl-CoA was shown to inhibit complex III. The inhibition was noncompetitive regarding reduced ubiquinone and uncompetitive regarding cytochrome *c*. In addition, octanoyl-CoA caused a blue shift in the *gamma* band of the absorption spectrum of reduced complex III. This effect may play a role in the pathogenesis of medium-chain and multiple acyl-CoA dehydrogenase deficiency, Reye syndrome, and Jamaican vomiting sickness which are inherited and acquired conditions of intracellular accumulation of octanoyl-CoA.

1. Introduction

Short- and medium-chain organic acids and acyl-CoAs are generated by the mitochondrial breakdown of fatty acids and some amino acids. Degradation of these metabolites generates energy substrates for tricarboxylic acid (TCA) cycle and respiratory chain. Beside catabolism they play an important role during anabolism by providing C-atom backbones for biosynthesis of a vast number of cellular components. Whereas amino acids or the corresponding *alpha*-keto acids cross the mitochondrial membrane via specific carrier systems and are further degraded, mitochondrial import of fatty acids involves a complex transport system. Long-chain fatty acidsare activated by acyl-CoA synthase and are converted in an acylcarnitine by carnitine palmitoyltranferase I, both steps taking place at the outer mitochondrial membrane [1]. The acylcarnitines are transported from mitochondrial intermembrane space into matrix by acylcarnitine translocase and, subsequently, are converted in an acyl-CoA by carnitine palmitoyltransferase II. This carnitine cycle may also function in a reverse mode when organic acids and acyl-CoAs accumulate in mitochondria [2] resulting in increased cellular excretion of acylcarnitines. Accumulation of short- and medium-chain organic acids, acylcarnitines, and acyl-CoAs (SMOAA) is a biochemical hallmark of organic acidurias such as glutaryl-CoA dehydrogenase (GCDH) deficiency or methylmalonic aciduria (MMA), *beta*-oxidation defects such as medium-chain acyl-CoA dehydrogenase deficiency or short-chain acyl-CoA dehydrogenase deficiency, Jamaican vomiting sickness (hypoglycin A toxicity due to poisoning with unripe ackee fruit), and Reye syndrome. It has been speculated that accumulating SMOAAs play a central role in the pathophysiology of these metabolic disorders by interfering with mitochondrial energy metabolism. This concept has been studied in detail for GCDH deficiency [3–5], propionic aciduria [6] and MMA [7–9]. However, a systematic approach investigating the impact of SMOAAs on mitochondrial energy metabolism considering chain length specificity is yet lacking. In contrast, several studies have investigated the impact of long-chain acyl-CoAs that accumulate in very long-chain

Abbreviations: CoA, coenzyme A; KGDHc, *alpha*-ketoglutarate dehydrogenase complex; PDHc, pyruvate dehydrogenase complex; DBH, decylubihydroquinone; TCA, tricarboxylic acid; SMOAA, short- and medium-chain organic acids, acylcarnitines, and acyl-CoAs; GCDH, glutaryl-CoA dehydrogenase; MMA, methylmalonic aciduria; A-CoA, Acetyl-CoA; P-CoA, propionyl-CoA; B-CoA, butyryl-CoA; I-CoA, isovaleryl-CoA; H-CoA, hexanoyl-CoA; O-CoA, octanoyl-CoA; M-CoA, malonyl-CoA; MM-CoA, methylmalonyl-CoA; G-CoA, glutaryl-CoA; SMPs, submitochondrial particles from bovine heart; AIC, Akaike's Information Criterion; MCAD, medium-chain acyl-CoA dehydrogenase; ETF, electron transfer flavoprotein

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acyl-CoA dehydrogenase deficiency and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency on mitochondrial enzymes and transporters. Long-chain acyl-CoAs revealed to be inhibitors of citrate synthase, glutamate dehydrogenase, and malate dehydrogenase (palmitoyl-CoA [[10,11]]), succinate-driven oxidative phosphorylation (palmitoyl-CoA [12]), mitochondrial dicarboxylate carrier (2,3-unsatured, 3-hydroxy-, 3-oxo-, palmitoyl-CoA [13]), ATP/ADP carrier (long-chain acyl-CoAs [13,14]), PDHc (palmitoyl-CoA [15]), rotenone-insensitive NADH-cytochrome *c* oxidoreductase (arachidonyl-CoA [16]), and NADH-cytochrome *c* oxidoreductase (oleoyl-CoA [17]). However, it has been hypothesized that prominent pathophysiologic features of disorders in the degradation of long-chain fatty acids are mainly caused by the primary lack of energy production, whereas in disorders of short- and medium-chain fatty acid degradation accumulating toxic metabolites are likely to induce secondary pathological changes [18]. To further systematically evaluate the pathophysiologic role of SMOAAs, we investigated their impact on central components of mitochondrial energy production, namely *alpha*-ketoglutarate dehydrogenase complex and pyruvate dehydrogenase complex – the rate limiting enzymes of TC cycle – as well as single enzyme complexes I–V of respiratory chain.

2. Material and methods

2.1. Reagents

All reagents were purchased from Sigma Aldrich (Taufkirchen, Germany). We studied the following SMOAAs:

Acylcarnitine: D,L-acetyl-, propionyl-, butyryl-, isovaleryl-, valeryl-, hexanoyl-, and octanoylcarnitine.
Organic acids: Acetic, propionic, butyric, isovaleric, valeric, hexanoic, malonic, methylmalonic, glutaric, and 3-hydroxyglutaric acid.
Acyl-CoAs: Acetyl (A-CoA)-, propionyl (P-CoA)-, butyryl (B-CoA)-, isovaleryl (I-CoA)-, hexanoyl (H-CoA)-, octanoyl (O-CoA)-, malonyl (M-CoA)-, methylmalonyl (MM-CoA)-, and glutaryl (G-CoA)-CoA.

2.2. Preparation of submitochondrial particles from bovine heart

Submitochondrial particles (SMPs) from bovine heart were prepared as previously described [19]. Protein content was determined according to Lowry et al. [20] with modifications [21] using bovine serum albumin as standard.

2.3. Spectrophotometric analysis of single enzyme complexes I–V of respiratory chain

Steady state activities of enzyme complexes were recorded using a computer tunable spectrophotometer (Spectramax Plus Microplate Reader, Molecular Devices, Sunnyvale, California, U.S.A.) operating in the dual wavelength mode. Samples were analyzed in temperature-controlled 96-well plates at a final volume of 300 µL. Catalytic activities of respiratory chain complexes I–V in SMPs were investigated as previously described. In brief, complex I activity was detected as NADH oxidation [19], complex II activity as dichloroindophenol reduction [22], complex III activity as cytochrome *c* reduction [23], complex IV as cytochrome *c* oxidation [24], and complex V activity as NADH reduction in the presence of phosphoenolpyruvate kinase and lactate dehydrogenase [25]. Addition of standard respiratory chain inhibitors (complex I: 2-*n*-decylquinazolin-4-yl-amine [1 µmol/L]; complex II: thenoyltrifluoroacetone [8 mmol/L]; complex III: antimycin A [1 µmol/L]; complex IV: NaCN [2 mmol/L]; complex V: oligomycin [80 µmol/L]) revealed good inhibitory responses (93–100% of control activity, $p < 0.001$ versus controls), confirming specific enzyme activity in our assay system. Complex III activity was also measured using purified bovine complex III, which was a kind gift of Prof. Ulrich Brandt (Gustav-Embden-Zentrum der Biologischen Chemie, Johann Wolfgang Goethe University, Frankfurt, Germany).

2.4. Spectral shift experiments

Spectral shift experiments were performed using purified complex III. Absorption spectra were recorded in a computer tunable spectrophotometer (Spectramax Plus Microplate Reader, Molecular Devices, Sunnyvale, California, USA) using microplates. Spectra of oxidized and reduced enzyme as well as reduced enzyme with a saturating amount of octanoyl-CoA were recorded. Complex III was reduced by adding 20 nmol sodium dithionite. When reduced spectrum was recorded in the presence of octanoyl-CoA, the enzyme was incubated first with the inhibitor in a buffer containing 250 mmol/L sucrose, 50 mmol/L KCl, 5 mmol/L $MgCl_2$, and 20 mmol/L Tris–HCl (total volume 100 µL, pH 7.4, 25 °C) and was subsequently reduced. Difference spectra were calculated by Softmax Pro Software defining oxidized or reduced spectrum as baseline.

2.5 Alpha-ketoglutarate dehydrogenase complex (KGDHc)

KGDHc activity was measured according to Humphries and Szweda [26] with modifications. KGDHc (650 mU/mL; purified from porcine heart, Sigma-Aldrich) was assayed in a buffer containing 35 mmol/L potassium phosphate, 5 mmol/L $MgCl_2$, 0.5 mmol/L EDTA, 0.5 mmol/L NAD, 0.2 mmol/L thiamine pyrophosphate, 0.04 mmol/L CoA-SH, and 2 mmol/L *alpha*-ketoglutarate which was adjusted to pH 7.4 (30°C). KGDHc activity was determined as NAD reduction at $\lambda = 340–400$ nm.

2.6. Pyruvate dehydrogenase complex (PDHc)

PDHc activity was investigated according to a recently described method [27]. In brief, PDHc (160 mU/mL; purified from porcine heart, Sigma-Aldrich) was assayed in a buffer containing 0.05 mol/L potassium phosphate, 2.5 mmol/L NAD, 0.2 mmol/L thiamine pyrophosphate, 0.1 mmol/L CoA, 0.1% Triton X-100, 1 mmol/L $MgCl_2$, 1 mg/mL bovine serum albumin, 0.6 mmol/L *p*-iodonitrotetrazolium violet, and 6.5 µmol/L phenazine methosulfate which was adjusted to pH 7.4 (25 °C). PDHc activity was determined as *p*-iodonitrotetrazolium violet reduction at $\lambda = 500–750$ nm.

2.7. Analyses of inhibition studies

Inhibition studies were analyzed using GraphPad prism 5.01 software for MS Windows. Only doubling or halving of enzyme activity by SMOAAs at a concentration of 1 mmol/L was considered as an effect of potential (patho-)physiological relevance.

3. Results

3.1. Octanoyl-CoA inhibits complex III

We investigated the impact of SMOAAs (each 1 mmol/L, in Tris–HCl pH 7.4) on single enzyme complexes I–V of respiratory chain using SMPs. The major effect was a strong and concentration-dependent inhibition of complex III activity by octanoyl-CoA (1 mmol/L octanoyl-CoA: 78% inhibition). Other acyl-CoAs had no inhibitory impact or were even activating (e.g. acetyl-CoA: complex I; butyryl-CoA, malonyl-CoA, complex III; Fig. 1). To exclude that the inhibition of complex III was an artifact caused by the preparation of SMPs, we also studied the inhibitory effect of octanoyl-CoA using purified complex III revealing similar results as shown for complex

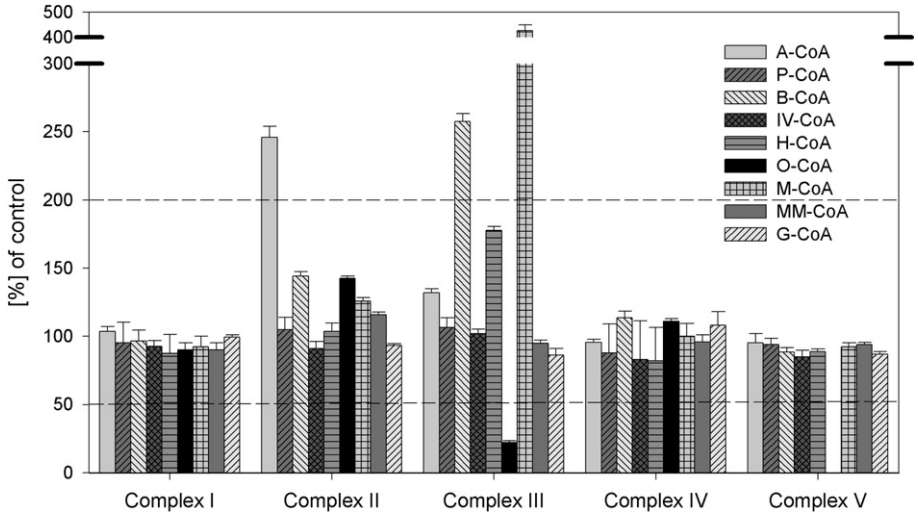

Fig. 1. Impact of short- and medium-chain acyl-CoAs (each 1 mmol/L, in Tris–HCl pH 7.4) on single enzyme complexes I–V of respiratory chain using SMPs. The major effect was a strong inhibition of complex III activity by octanoyl-CoA (1 mmol/L octanoyl-CoA: 78% inhibition). Other acyl-CoAs had no inhibitory impact or were even activating (e.g. acetyl-CoA: complex II; butyryl-CoA, malonyl-CoA: complex III). Short dashed lines indicate 50% and 200% of control activity. Activities are given as percent of control. Data are expressed as mean ±S.D., experiments were performed in triplicates.

III in SMPs. In addition, we repeated the experiment with different charges of octanoyl-CoA from Sigma-Aldrich and Larodan Fine Chemicals to rule out that the inhibition was caused by contaminations or by-products of CoA-ester synthesis. However, the inhibitory effect of octanoyl-CoA on complex III was the same in each experimental series.

Neither organic acids nor acylcarnitines affected the activity of any respiratory chain complex (Table 1) except for the well-known complex II inhibitor malonate. We also investigated the impact of octanoic acid on complex III to eliminate the possibility that octanoyl-CoA was degraded by dissolving and the free organic acid inhibits complex III. However, octanoic acid did not influence enzyme activity (data not shown).

Table 1
Impact of short- and medium-chain acylcarnitines and organic acids on single enzyme complexes I–V of respiratory chain

	Complex I	Complex II	Complex III	Complex IV	Complex V
	M±SD (%)	M±SD (%)	M±SD (%)	M±SD (%)	M±SD (%)
l-Acetyl-carnitine	102±1	87±1	105±5	74±5	92±3
Propionyl-carnitine	104±5	95±3	74±5	76±4	92±4
Butyryl-carnitine	107±1	94±4	110±3	89±2	98±2
Isovaleryl-carnitine	84±3	85±3	84±4	97±3	101±3
Valeryl-carnitine	87±3	92±2	83±15	94±4	98±1
Hexanoyl-carnitine	89±3	88±1	94±9	118±19	100±2
Octanoyl-carnitine	93±10	85±2	105±14	96±6	99±1
d-Acetyl-carnitine	98±3	91±1	99±3	110±2	73±4
Acetic acid	92±9	98±5	91±2	99±9	101±3
Propionic acid	114±0	88±5	102±8	105±3	95±4
Butyric acid	111±11	80±8	86±11	93±2	107±95
Isovaleric acid	108±10	80±7	87±1	108±2	111±3
Valeric acid	106±2	84±2	88±3	98±5	99±4
Hexanoic acid	103±13	76±2	95±3	103±1	99±1
Malonic acid	97±6	19±2	102±3	103±5	100±8
Methylmalonic acid	103±15	89±7	96±6	101±2	96±2
Glutaric acid	96±7	99±3	98±4	96±4	94±3
3-Hydroxyglutaric acid	93±2	103±3	99±11	98±4	94±1
Octanoic acid			110±9		

Neither the organic acids nor the acylcarnitines (each 1 mmol/L, in Tris–HCl pH 7.4) investigated in this study affected the activity of any respiratory chain complex except for the well-known complex II inhibitor malonate. Activities are given as percent of control. Data are expressed as mean±S.D. (n=4).

3.2. Mode of complex III inhibition by octanoyl-CoA

To further clarify the mode of complex III inhibition by octanoyl-CoA, we performed inhibition studies with varying concentrations of octanoyl-CoA (125, 250, 500, 750, and 1000 μmol/L), DBH (1, 5, 12.5, 25, and 50 μmol/L), and cytochrome c (4, 7.5, 15, 30, and 60 μmol/L). The inhibition was concentration-dependent (octanoyl-CoA, 0.05, 0.125, 0.25, 0.5, 0.5, 1, 2, and 3 mmol/L; Fig. 2A). Nonlinear regression analysis revealed that octanoyl-CoA-induced inhibition was noncompetitive regarding DBH (V_{max}: 2.9±0.7 U/mg, K_m: 44.8±19 μmol/L, K_i: 728±74.7 μmol/L; difference in AICs: noncompetitive vs. competitive, −1.6; noncompetitive vs. uncompetitive, −12.5; noncompetitive vs. mixed model inhibition, −2.6) and uncompetitive regarding cytochrome c (V_{max}: 2.9±0.7 U/mg, K_m: 26.3±13.49 μmol/L, αK_i: 184.0±27.8 μmol/L; difference in AICs: uncompetitive vs. noncompetitive, −12.1; uncompetitive vs. competitive, −32.6; mixed model inhibition ambiguous) (Fig. 2B, C) indicating that octanoyl-CoA does not directly interact with DBH or cytochrome c binding site.

3.3. Effects of octanoyl-CoA on the absorption spectrum of complex III

When octanoyl-CoA was added to reduce complex III, it had a hypsochromic effect and caused a blue shift in the *gamma* band of the absorption spectrum of reduced complex III. The blue shift resulted in a difference spectrum with a maximum at 412 nm and a minimum at 431 nm. This effect was concentration-dependent and, thus, higher concentrations of octanoyl-CoA intensified the blue shift. The *alpha* band did not show a blue shift but a minimum at 561 nm. The cytochrome c peak at 555 nm was less affected. The effect of octanoyl-CoA on reduced absorption spectrum of complex III suggests that octanoyl-CoA inhibits the reduction of cytochrome b (Fig. 3A). Difference spectra of oxidized and in the presence of an inhibitor reduced enzyme further underline this suggestion. Increasing amounts of octanoyl-CoA caused a concentration-dependent decrease of cytochrome b peak in the *alpha* band at 560 nm, whereas reduction of cytochrome c peak at 555 nm was less reduced (Fig. 3B). In summary, spectral data indicate that octansoyl-CoA reduces complex III activity by inhibiting the reduction of cytochrome b. To exclude a general effect of medium-chain acyl-CoAs, we also incubated reduced complex III with hexanoyl-CoA. In contrast to octanoyl-CoA, this acyl-CoA did not affect the absorption spectrum of complex III (data not shown).

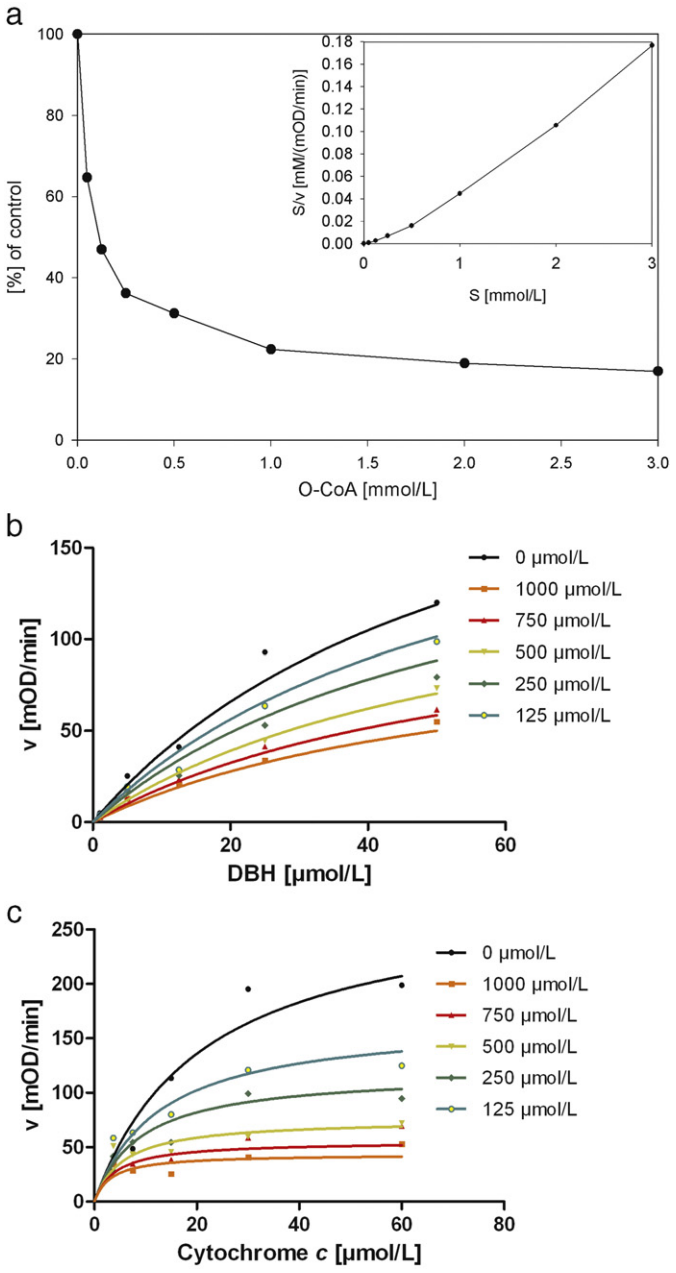

Fig. 2. Mode of complex III inhibition by octanoyl-CoA. Inhibition studies were performed with varying concentrations of octanoyl-CoA (125, 250, 500, 750, and 1000 µmol/L), DBH (1, 5, 12.5, 25, and 50 µmol/L) and cytochrome *c* (4, 7.5, 15, 30, and 60 µmol/L). Inhibition was concentration-dependent (a). Nonlinear regression analysis revealed that the inhibition was noncompetitive regarding DBH (V_{max}: 2.9±0.7 U/mg, K_m: 44.8±19 µmol/L, K_i: 728± 74.7 µmol/L; (b) and uncompetitive regarding cytochrome *c* (V_{max}: 2.9±0.7 U/mg, K_m: 26.3 ± 13.49 µmol/L, *alpha*-K_i: 184.0±27.8 µmol/L; (c). Activities are given as mOD/min. All data are expressed as mean, experiments were performed in triplicates.

3.4. Impact of short- and medium-chain acyl-CoAs on pyruvate dehydrogenase complex (PDHc)

We investigated the impact of SMOAAs (each 250, 500, and 1000 µmol/L, in Tris–HCl, adjusted to pH 7.4) on PDHc activity. All investigated acyl-CoAs inhibited PDHc activity but by a varying degree depending on chain length and number of carboxylic groups (Fig. 4). Short-chain monocarboxylic acyl-CoAs revealed the strongest inhibitory impact on PDHc activity. Medium-chain and dicarboxylic acyl-CoAs were less effective inhibitors. Neither acylcarnitines nor organic acids that were investigated had an influence on enzyme activity (Table 2).

3.5. Impact of short- and medium-chain acyl-CoAs on alpha-ketoglutarate dehydrogenase complex (KGDHc)

Next, we studied the impact of SMOAAs (each 250, 500, and 1000 µmol/L, in Tris–HCl, adjusted to pH 7.4) on KGDHc activity. In analogy to the inhibition studies using PDHc, all investigated acyl-CoAs inhibited KGDHc activity depending on chain length and number of carboxylic groups (Fig. 5). Unbranched chain dicarboxylic and short-chain monocarboxylic acyl-CoAs revealed the strongest inhibitory impact on KGDHc activity. Unbranched medium-chain monocarboxylic acyl-CoAs and branched-chain mono- and dicarboxylic acids were less effective. All investigated acylcarnitines inhibited KGDHc activity by about 25%. Organic acids did not affect enzyme activity (Table 2).

4. Discussion

The aim of this study was to investigate the impact of SMOAAs on central components of the mitochondrial energy metabolism namely

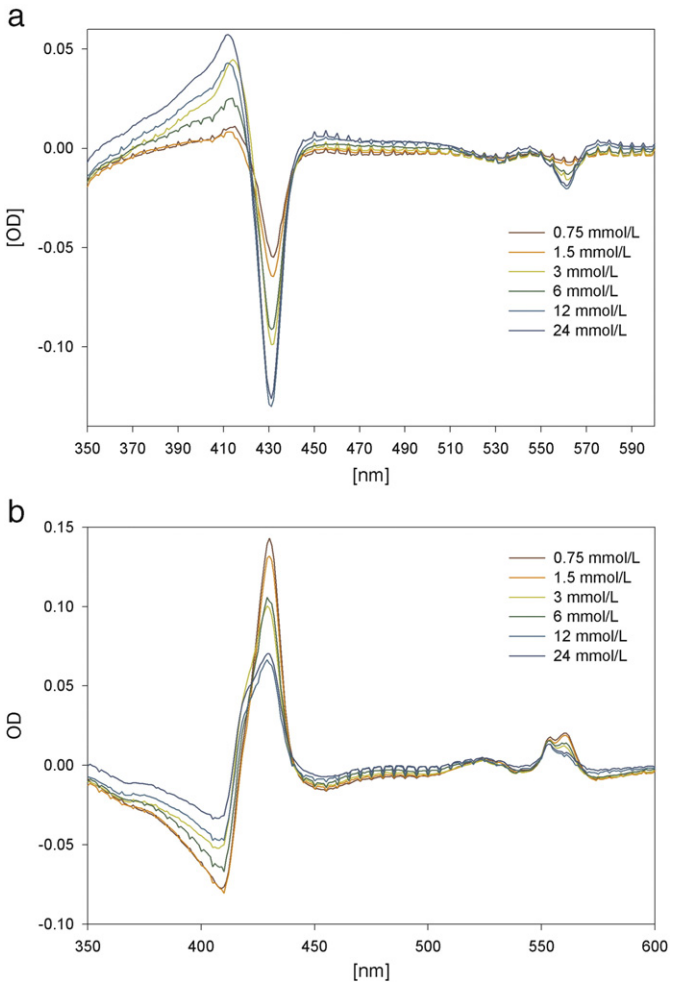

Fig. 3. Effect of octanoyl-CoA on absorption spectrum of complex III. Incubation of reduced complex III with octanoyl-CoA caused a blue shift in the *gamma* band of the absorption spectrum of reduced complex III with a maximum at 412 nm and a minimum at 431 nm. This effect was concentration-dependent and higher octanoyl-CoA concentrations intensified the blue shift. The *alpha* band did not show a blue shift but a minimum at 561 nm that is likely to refer to cytochrome b_{562} (A). Difference spectra of oxidized and in presence of an inhibitor reduced enzyme displayed that increasing amounts of octanoyl-CoA cause a concentration-dependent decrease in the *alpha* band at 560 nm (cytochrome b_{562} peak), whereas the cytochrome *c* peak at 555 nm was less affected (B). These findings suggest that octanoyl-CoA inhibits the reduction of cytochrome b_{562}.

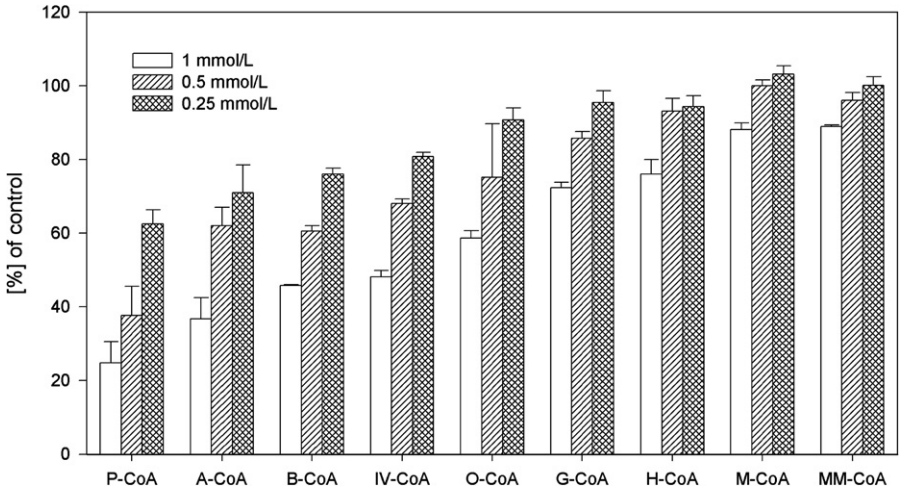

Fig. 4. Impact of short- and medium-chain acyl-CoAs (each 250, 500, and 1000 μmol/L, in Tris–HCl, adjusted to pH 7.4) on PDHc activity. All investigated acyl-CoAs inhibited PDHc activity. The inhibitory effect was critically dependent on chain length and number of carboxylic groups. Short-chain monocarboxylic acyl-CoAs revealed the strongest inhibitory effect on PDHc activity. Medium-chain and dicarboxylic acyl-CoAs were less effective inhibitors. Activities are given as percent of control. All data are expressed as mean ± S.D., experiments were performed in triplicates.

KGDHc, PDHc, and single enzyme complexes I–V of the respiratory chain. All investigated acyl-CoAs had an inhibitory impact on PDHc and KGDHc activity. However, effect sizes were critically dependent on chain length and number of functional groups. In previous studies, we have shown that propionyl-CoA and glutaryl-CoA uncompetitively inhibit both PDHc and KGDHc. The most likely mechanism is product feedback inhibition of the E2 subunits [4,6]. Due to the fact that increasing structural similarity to glutaryl-CoA or propionyl-CoA resulted in increasing inhibitory potential on the respective enzyme activity, it seems likely that the inhibitory effect of other acyl-CoAs is based on the same mechanism. In contrast, all organic acids investigated in this study had no effect on PDHc or KGDHc activity. Whereas the corresponding acylcarnitines did not also affect PDHc activity, they showed a general and small inhibitory impact on KGDHc activity.

Next, we investigated the impact of SMOAAs on single enzyme complexes I–V of the respiratory chain. All studied acylcarnitines and organic acids (except for malonic acid) did not affect the activity of respiratory chain complexes. Again, only a few acyl-CoAs revealed an impact on enzyme activities. Malonyl-CoA and butyryl-CoA induced an activation of complex III activity, and acetyl-CoA activated complex II. Previous studies have shown that complex II and complex III activity can be activated by specific phospholipids [28–31]. In addition, activation of complex II by succinyl-CoA was demonstrated [32]. The above described activating impact of acetyl-CoA, malonyl-CoA, and butyryl-CoA may base on a similar mechanism.

However, the most intriguing finding of our study was the inhibition of complex III by octanoyl-CoA. Octanoyl-CoA apparently displays no structural similarity to the substrates ubiquinone and cytochrome *c* or any known inhibitor of complex III. Consistently, competition experiments with varying substrate concentrations revealed that the inhibition was *noncompetitive* regarding DBH, i.e. the inhibitor reversibly binds to both the enzyme–substrate complex and the enzyme itself, and *uncompetitive* regarding cytochrome *c*, i.e. the inhibitor binds to the enzyme–substrate complex, but not the free enzyme. Therefore, binding of cytochrome *c* but not binding of DBH to complex III seems to be obligatory for the inhibition by octanoyl-CoA. This finding may be explained by the fact that binding of cytochrome *c* is obligatory for the electron flow of the high-potential chain and, thereby, catalytic activity of the enzyme.

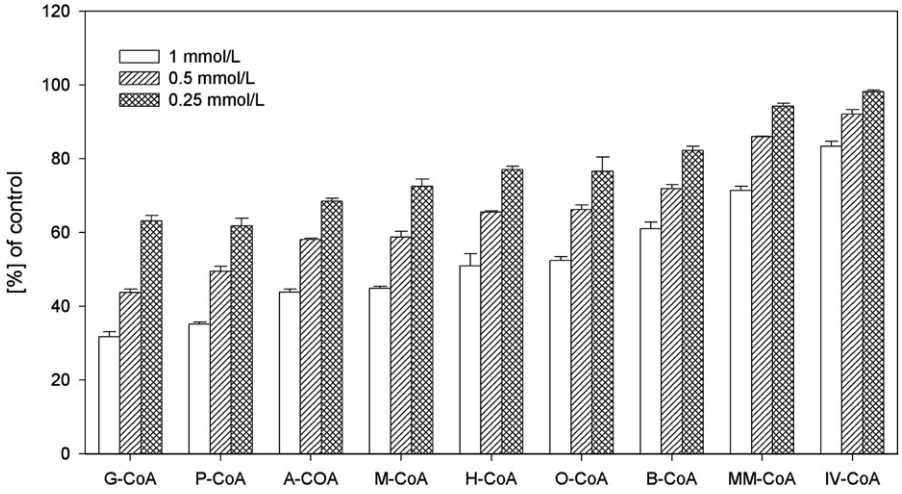

Fig. 5. Impact of short- and medium-chain acyl-CoAs (each 250, 500, and 1000 μmol/L, in Tris–HCl, adjusted to pH 7.4) on KGDHc activity. All investigated acyl-CoAs inhibited KGDHc activity depending on chain length and number of carboxylic groups. Unbranched chain dicarboxylic and short-chain monocarboxylic acyl-CoAs revealed the strongest inhibitory impact on KGDHc activity. Unbranched medium-chain monocarboxylic acyl-CoAs and branched-chain mono- and dicarboxylic acid were less effective inhibitors. Activities are given as percent of control. All data are expressed as mean ± S.D., experiments were performed in triplicates.

Table 2
Impact of short- and medium-chain acylcarnitines and organic acids on PDHc and KGDHc activity

	PDHc	KGDHc
	M (%)±SD (%)	M (%)±SD (%)
l-Acetyl-carnitine	87±3	76±2
Propionyl-carnitine	94±2	76±1
Butyryl-carnitine	98±1	78±1
Isovaleryl-carnitine	105±5	78±2
Valeryl-carnitine	97±1	78±2
Hexanoyl-carnitine	115±4	85±3
Octanoyl-carnitine	101±2	70±3
d-Acetyl-carnitine	99±1	85±7
Acetic acid	110±4	101±1
Propionic acid	104±6	99±1
Butyric acid	119±2	102±6
Isovaleric acid	104±4	101±2
Valeric acid	99±17	96±1
Hexanoic acid	101±2	95±3
Malonic acid	113±3	99±0
Methylmalonic acid	117±6	101±4
Glutaric acid	106±7	94±2
3-Hydroxyglutaric acid	112±3	89±2

Neither organic acids nor acylcarnitines (each 1 mmol/L, in Tris–HCl pH 7.4) affected the activity of PDHc. All investigated acylcarnitines inhibited KGDHc activity by about 25%. Organic acids did not affect enzyme activity. Activities are given as percent of control. Data are expressed as mean±S.D. (n=4).

In addition, octanoyl-CoA induces a blue shift of the *gamma* band in the absorption spectrum of reduced complex III in analogy to funicolsin [33] and ilicicolin H [34] both inhibiting ubiquinone reduction at center N (mitochondrial matrix directed [35]). The blue shift of the *gamma* band indicates that reduction of cytochrome *c* (peak at 415 nm) and cytochrome *b* (peak at 429 nm) was inhibited. The *alpha* band displayed a minimum at 561 nm that is likely to refer to heme b_{562}. The cytochrome *c* peak at 555 nm was less affected. Difference spectra of oxidized and in presence of an inhibitor reduced enzyme showed a strong decrease of cytochrome *b* peak in the alpha band at 560 nm and only a mild reduction of cytochrome *c* peak at 555 nm. These observations suggest that the inhibition of complex III activity by octanoyl-CoA is mediated by an inhibited reduction of heme b_{562} that is located at center N [36]. Therefore, we postulate that the inhibitory impact of octanoyl-CoA is based on an interaction with center N of complex III.

Octanoyl-CoA pathophysiologically accumulates in medium-chain acyl-CoA dehydrogenase (MCAD) deficiency but the precise intracellular, and particularly hepatic, concentration of this metabolite is yet unknown. The only study investigating the hepatic acyl-CoA profiles in MCAD deficiency did not provide data for octanoyl-CoA [37]. Though MCAD deficiency is the most frequent error of fatty acid oxidation [38] with an estimated frequency of 1:6,000 to 1:10,000 Caucasian births, the pathophysiology of this disorder is yet poorly understood. During late stages of fasting, fatty acids are used for hepatic ketone synthesis and to provide 80% of total body energy demands. Patients with MCAD deficiency have a mainly hepatic type of presentation which is precipitated by episodes that are likely to induce catabolic state, such as febrile illness, vomiting, and encephalopathy [39–41]. Approximately 25% of untreated patients have died suddenly and unexpectedly, whereas a similar percentage of patients may remain free of symptoms throughout life. During catabolic state, the manifestation of severe symptoms such as lethargy and nausea develops in association with the marked increase in fatty acids, whereas ketone bodies remain low [39,42]. Hypoglycemia may develop shortly thereafter; however, it should be stressed that patients may become dangerously ill before hypoglycemia occurs. This suggests that toxic effects of elevated plasma free fatty acids or intracellularly accumulating acyl-CoA esters might be involved in the pathogenesis of MCAD deficiency—in addition to hypoketotic hypoglycemia. Interestingly, electron microscopical studies of hepatic mitochondria from MCAD deficient patients during metabolic decompensation revealed characteristic features of a severely disturbed mitochondrial energy metabolism [43–47].

Elevated concentrations of octanoyl-CoA are also found in three additional conditions, i.e. multiple acyl-CoA dehydrogenase deficiency, Reye syndrome, and Jamaican vomiting sickness. Multiple acyl-CoA dehydrogenase deficiency is caused by inherited deficiency of electron transfer flavoprotein (ETF) or ETF-Q causing loss or reduced activity of all mitochondrial FAD-dependent acyl-CoA dehydrogenases [48,49]. Due to the significant impairment of energy metabolism, multiple organ failure is frequently found. The etiology of Reye syndrome, a combination of liver disease and noninflammatory encephalopathy with sudden onset [50], is unknown but a strong epidemiologic association to the ingestion of acetylaspartic acid during antecendent varicella or influenza-like illnesses has been shown [51–54] although not undoubted [55,56]. Multiple site inhibition of mitochondrial enzymes of energy metabolism has been considered which is supported by the finding of disrupted mitochondrial cristae [57], the sites of membrane-associated acyl-CoA dehydrogenases of branched-chain and fatty acid oxidation [58,59]. Jamaican vomiting sickness is peculiar to Jamaica and is caused by inhibition of FAD-dependent acyl-CoA dehydrogenases by hypoglycin A intoxication following ingestion of unriped Ackee fruit [60]. Fatty change of the liver, kidneys, and other organs is also a prominent feature in this intoxication which has a high mortality rate. In addition to octanoyl-CoA, other short- and medium-chain acyl-CoAs are found to be elevated in these conditions [37,61].

Although impairment of mitochondrial energy metabolism by accumulating short- and medium-chain acyl-CoAs – especially octanoyl-CoA–may not be the first step in the pathogenesis of MCAD deficiency, multiple acyl-CoA dehydrogenase deficiency, Reye syndrome, and Jamaican vomiting sickness, it seems likely to assume that they act synergistically with other toxic metabolites inducing a secondary severe inhibition of key enzymes of mitochondrial energy metabolism resulting in multiorgan failure or even death if untreated.

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