

Rapid Report

β -Oxidation of 5-hydroxydecanoate, a putative blocker of mitochondrial ATP-sensitive potassium channels

Peter J. Hanley, K. V. Gopalan*, Rachel A. Lareau*, D. K. Srivastava*, Martin von Meltzer† and Jürgen Daut

Institut für Normale und Pathologische Physiologie, Universität Marburg, Deutschhausstrasse 2, 35037 Marburg, Germany, *Department of Biochemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105, USA and †Fachbereich Chemie, Universität Marburg, Hans-Meerwein-Strasse, 35032 Marburg, Germany

5-Hydroxydecanoate (5-HD) inhibits ischaemic and pharmacological preconditioning of the heart. Since 5-HD is thought to inhibit specifically the putative mitochondrial ATP-sensitive K^+ (K_{ATP}) channel, this channel has been inferred to be a mediator of preconditioning. However, it has recently been shown that 5-HD is a substrate for acyl-CoA synthetase, the mitochondrial enzyme which 'activates' fatty acids. Here, we tested whether activated 5-HD, 5-hydroxydecanoyl-CoA (5-HD-CoA), is a substrate for medium-chain acyl-CoA dehydrogenase (MCAD), the committed step of the mitochondrial β -oxidation pathway. Using a molecular model, we predicted that the hydroxyl group on the acyl tail of 5-HD-CoA would not sterically hinder the active site of MCAD. Indeed, we found that 5-HD-CoA was a substrate for purified human liver MCAD with a K_m of $12.8 \pm 0.6 \mu M$ and a k_{cat} of $14.1 s^{-1}$. For comparison, with decanoyl-CoA ($K_m \sim 3 \mu M$) as substrate, k_{cat} was $6.4 s^{-1}$. 5-HD-CoA was also a substrate for purified pig kidney MCAD. We next tested whether the reaction product, 5-hydroxydecanoyl-CoA (5-HD-enoyl-CoA), was a substrate for enoyl-CoA hydratase, the second enzyme of the β -oxidation pathway. Similar to decenoyl-CoA, purified 5-HD-enoyl-CoA was also a substrate for the hydratase reaction. In conclusion, we have shown that 5-HD is metabolised at least as far as the third enzyme of the β -oxidation pathway. Our results open the possibility that β -oxidation of 5-HD or metabolic intermediates of 5-HD may be responsible for the inhibitory effects of 5-HD on preconditioning of the heart.

(Received 4 December 2002; accepted after revision 27 January 2003; first published online 31 January 2003)

Corresponding author J. Daut: Institut für Normale und Pathologische Physiologie, Universität Marburg, Deutschhausstrasse 2, 35037 Marburg, Germany. Email: daut@mail.uni-marburg.de

Preconditioning of the heart by means of brief (~ 5 min) periods of ischaemia or various pharmacological agents, such as diazoxide and volatile anaesthetics, reduces the cellular damage which follows a prolonged ischaemic insult (Cope *et al.* 1997; Schulz *et al.* 2001). Mitochondrial K_{ATP} channels are thought to play a central role in mediating these phenomena (Gross & Fryer, 1999; Gross, 2000; Sato & Marbán, 2000). An important piece of evidence for implicating mitochondrial K_{ATP} channels as mediators of preconditioning is the consistent inhibitory effect of 5-hydroxydecanoate (5-HD). This drug, originally investigated for its antiarrhythmic actions (Notsu *et al.* 1992), has been shown to inhibit both ischaemic and pharmacological preconditioning (Gross & Fryer, 1999; Gross, 2000; Sato & Marbán, 2000). Hence, elucidation of the molecular action(s) of 5-HD may provide a clue to the elusive mechanism of preconditioning. Garlid *et al.* (1997) reported that 5-HD inhibited K^+ flux via diazoxide-activated mitochondrial K_{ATP} channels. In further work,

Jaburek *et al.* (1998) found that 5-HD inhibited K^+ flux via mitochondrial K_{ATP} channels in heart and liver mitochondria with a half-maximal inhibitory concentration of 45–75 μM . These studies have fostered the notion that 5-HD is a specific inhibitor of the mitochondrial K_{ATP} channel.

Recently, Hanley *et al.* (2002a) have shown that 5-HD is a substrate for acyl-CoA synthetase, the mitochondrial enzyme which thioester-links fatty acids with coenzyme A (CoA), a reaction referred to as fatty acid 'activation'. This observation opened the possibility that the activated form of 5-HD, 5-HD-CoA (5-hydroxydecanoyl-CoA), might be responsible for inhibiting preconditioning. By analogy with other acyl-CoA esters, the possible targets of 5-HD-CoA include K_{ATP} channels, protein kinase C and the ADP/ATP translocase (Paucek *et al.* 1996; Knudsen *et al.* 1999; Liu *et al.* 2001). Another possibility is that 5-HD-CoA may serve as a substrate for medium-chain acyl-CoA

dehydrogenase (MCAD), the enzyme which catalyses the initial oxidative step of mitochondrial β -oxidation. The structure and catalytic mechanism of MCAD have been extensively investigated. X-ray crystallographic structures of both pig liver and human liver MCAD have been determined at 2.4 and 2.75 Å (0.24 and 0.275 nm) resolution, respectively (Kim *et al.* 1993; Lee *et al.* 1996). These structural data revealed that the substrate-binding pocket, which is lined with hydrophobic amino acid residues, extends almost to the centre of the protein. Substrate binds to the oxidised enzyme, forming an enzyme–substrate complex, and two reducing equivalents are transferred to the bound FAD moiety (dehydrogenation step), yielding the enoyl-CoA product (for review, see Thorpe & Kim, 1995). The product dissociates after two molecules of electron transfer protein (ETF), the natural electron acceptor for MCAD (Roberts *et al.* 1996), have re-oxidised the enzyme. ETF transfers electrons to ubiquinone, thereby linking MCAD with the respiratory chain.

In the present study, we show that MCAD catalyses the conversion of 5-hydroxydecanoyl-CoA to 5-HD-enoyl-CoA. We further show that 5-HD-enoyl-CoA is a substrate for the second enzyme of the β -oxidation pathway, enoyl-CoA hydratase. Since the reaction rates were comparable to those of decanoate derivatives, our results raise the possibility that 5-HD may be completely oxidised in mitochondria. These findings have some important implications for our understanding of the mechanisms underlying ischaemic or pharmacological preconditioning.

METHODS

Preparation of 5-hydroxydecanoyl-CoA

5-Hydroxydecanoyl-CoA (5-HD-CoA) was synthesised using commercially available acyl-CoA synthetase (EC 6.2.1.3; Sigma). 5-Hydroxydecanoate (RBI, Sigma), coenzyme A and Na₂ATP were added to Tris buffer solution containing (mM): 100 Tris, 9.1 EDTA and 1.8 EDTA (pH adjusted to 7.5 with HCl). Coenzyme A concentration before and after the reaction was determined using DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), which reacts with free thiol groups and generates 2-nitro-5-thiobenzoate (peak absorbance, 412 nm). 5-HD-CoA was separated using a Waters HPLC system which incorporated an Alltech C₁₈-column (0.1 cm × 25 cm, 10 μm, Econosil). Methanol and 20 mM potassium phosphate solution (pH 7.0) were used as solvents. Electrospray ionisation mass spectrometry was used to confirm that the isolated peak was 5-HD-CoA (Hanley *et al.* 2002a).

Medium-chain acyl-CoA dehydrogenase

Recombinant human liver MCAD (medium-chain acyl-CoA dehydrogenase; EC 1.3.99.3) was expressed and purified as previously described (Peterson *et al.* 1995), whereas pig kidney MCAD was extracted and purified from tissue (Johnson *et al.* 1992). All experiments were performed at 25 °C in buffer solution containing (mM): 50 potassium phosphate, 0.3 EDTA and 10 % glycerol (pH 7.6). Activity of MCAD was routinely assayed by monitoring reduction of ferricinium hexafluorophosphate (FcPF₆) at 300 nm ($\epsilon = 4.3 \text{ mM}^{-1} \text{ cm}^{-1}$) using a Perkin-Elmer

Lambda 3B spectrophotometer. The cuvette had a path length of 1 cm and contained a magnetic stirrer. Decanoyl-CoA ($\epsilon = 4.3 \text{ mM}^{-1} \text{ cm}^{-1}$ at 259 nm) or 5-HD-CoA (ϵ assumed to be the same as for decanoyl-CoA) was used as substrate.

Molecular model of 5-hydroxydecanoyl-CoA bound to MCAD

A molecular model was used to depict both (*R*)- and (*S*)-5-HD-CoA bound to pig liver medium-chain acyl-CoA dehydrogenase. The X-ray crystallographic structure of this enzyme in the absence (pdb3mdd.pdb) and presence (pdb3mde.pdb) of octenoyl-CoA was downloaded from the Brookhaven Protein Data Bank. MCAD-bound octenoyl-CoA (C₈-CoA) was modified to decanoyl-CoA (C₁₀-CoA) and energy minimisation of the enzyme–C₁₀-CoA complex was performed as previously described (Peterson *et al.* 2001). A hydroxyl group was added in either the (*R*)- or (*S*)-configuration at position C-5 to generate a model of a MCAD-5-HD-CoA complex and to predict whether the hydroxyl group could sterically hinder catalysis. Amino acid residues located within 4 Å of the ligand were identified. Data were displayed on a Silicon Graphics Indigo XZ workstation.

Enoyl-CoA hydratase

The enoyl-CoA esters decenoyl-CoA and 5-hydroxydecanoyl-CoA (5-HD-enoyl-CoA) were prepared enzymatically using MCAD and FcPF₆ as previously described (Kumar & Srivastava, 1994) and purified via preparative HPLC. Purified 5-HD-enoyl-CoA was not metabolised by MCAD, indicating that it was not contaminated with 5-hydroxydecanoyl-CoA. Commercially available bovine liver enoyl-CoA hydratase (Fluka) was added to standard buffer solution and enzyme activity, using either decenoyl-CoA (K_m 300 μM; Waterson & Hill, 1972) or 5-HD-enoyl-CoA as substrate, was monitored by measuring absorbance at 263 nm (Waterson & Hill, 1972).

RESULTS

Molecular model of (*S*)-5-hydroxydecanoyl-CoA bound to MCAD

We have studied the question whether 5-HD-CoA can be metabolised by β -oxidation in the same way as decanoyl-CoA, a natural substrate of MCAD. In order to predict whether the hydroxyl group of 5-HD-CoA, which can have either an (*R*)- or (*S*)-configuration, could hinder catalysis, we incorporated 5-HD-CoA in a molecular model of pig liver MCAD. Figure 1A shows (*S*)-5-hydroxydecanoyl-CoA ((*S*)-5-HD-CoA) bound to a subunit of the homotetrameric enzyme MCAD. The atoms of (*S*)-5-HD-CoA are colour-coded whereas the protein is shown as a yellow ribbon. We identified the amino acid residues located within 4 Å (solid yellow structures) of the ligand (Fig. 1B). Glu-376 (labelled in Fig. 1B) is the active site base which abstracts the α -proton of the acyl-CoA substrate. After the α -proton is abstracted, the β -hydrogen is transferred to the N-5 position of the isoalloxazine ring of FAD (flavin adenine dinucleotide). The model suggests that the hydroxyl group of 5-HD-CoA, whether in the (*R*)- (not illustrated) or (*S*)-configuration (Fig. 1B), can be easily accommodated by the ligand-binding pocket of MCAD. Furthermore, the model indicates that 5-HD-CoA would not sterically hinder the active site.

5-Hydroxydecanoyl-CoA is a substrate for MCAD

Based on our above model and the known broad substrate specificity of MCAD (Peterson *et al.* 1995), we predicted that 5-HD-CoA would be a potential substrate for MCAD. In the experiments shown in Fig. 2, 5-HD-CoA was freshly synthesised using acyl-CoA synthetase, which was subsequently removed by ultrafiltration. The amount of 5-HD-CoA formed was determined by measuring the change in coenzyme A (CoA) concentration. Figure 2 shows that 5-HD-CoA was indeed a substrate for MCAD. In fact, maximal turnover rates of both liver (Fig. 2A and B) and kidney (Fig. 2C and D) MCAD were higher

when 5-HD-CoA was used as substrate compared to decanoyl-CoA. In the case of liver MCAD, the apparent k_{cat} was 10.3 s^{-1} with 5-HD-CoA as substrate (Fig. 2A) and 6.4 s^{-1} with decanoyl-CoA as substrate (Fig. 2B). The latter value is in line with the value of 7.4 s^{-1} reported by Peterson *et al.* (1995). Similar results were obtained using kidney MCAD: the apparent k_{cat} was 10.7 s^{-1} with 5-HD-CoA (Fig. 2C) and $k_{\text{cat}} 7.8 \text{ s}^{-1}$ with decanoyl-CoA as substrate (Fig. 2D). In accordance with these results, Peterson *et al.* (1995) reported a k_{cat} value of 8.6 s^{-1} with decanoyl-CoA as substrate for MCAD.

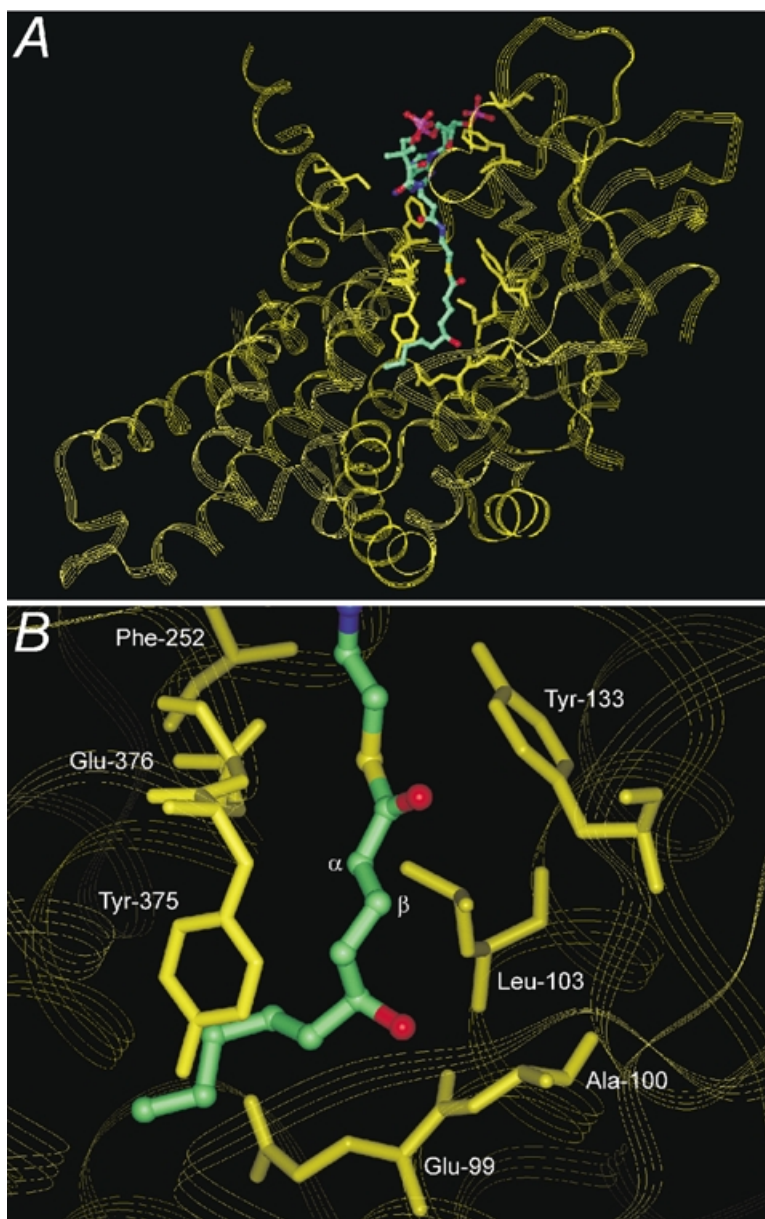


Figure 1. Molecular model of MCAD-(S)-5-hydroxydecanoyl-CoA complex

A, structure of pig liver MCAD monomer in complex with modelled ligand (S)-5-HD-CoA. Amino acid residues within 4 Å of the ligand are shown (solid yellow structures). B, enlarged view of the MCAD-(S)-5-HD-CoA complex showing relation of the (S)-5-hydroxyl group to labelled residues in the fatty acyl binding pocket. Glu-376 is the active site base which abstracts the α -proton from the acyl-CoA substrate whereas the β -hydrogen is transferred to FAD (not depicted).

Kinetics of 5-HD-CoA interaction with MCAD

The kinetics of human liver MCAD interacting with the acyl-CoA substrates octanoyl-CoA and decanoyl-CoA have been characterized (Peterson *et al.* 1995). In order to determine the kinetics of human liver MCAD interacting with 5-HD-CoA we purified 5-HD-CoA by preparative HPLC. Figure 3A shows the rate of MCAD-catalysed reaction as a function of [5-HD-CoA]. 5-HD-CoA was added from a stock solution of 2.6 mM in potassium phosphate (20 mM) solution (pH 7.0). The K_m was $12.8 \pm 0.6 \mu\text{M}$ and V_{max} (maximal rate) was $23.6 \mu\text{M s}^{-1}$ (k_{cat} , 14.1 s^{-1}). Data were fitted using the Michaelis-Menten equation, $V = V_{\text{max}} [S]/([S] + K_m)$, where V is rate and $[S]$ is substrate concentration. For comparison, with decanoyl-CoA as substrate, the K_m was found to be 4-fold lower ($\sim 3 \mu\text{M}$; Peterson *et al.* 1995), whereas the k_{cat} was about half (6.4 s^{-1} ; Fig. 2B).

The enoyl-CoA product 5-hydroxydecanoyl-CoA is a substrate for enoyl-CoA hydratase

Dehydrogenation of 5-HD-CoA via MCAD yields the enoyl-CoA product 5-hydroxydecanoyl-CoA (5-HD-enoyl-CoA). We next tested whether purified 5-HD-enoyl-CoA was a substrate for enoyl-CoA hydratase, the second enzyme of the mitochondrial β -oxidation pathway. Enzyme activity was monitored by measuring absorbance

at 263 nm (Waterson & Hill, 1972). When $85 \mu\text{M}$ decanoyl-CoA was added to $12 \mu\text{M}$ bovine liver hydratase, the enzyme activity was 0.021 min^{-1} (Fig. 3B). Similarly, with 5-HD-enoyl-CoA as substrate (added from a 2.2 mM stock solution), enoyl-CoA hydratase activity was 0.018 min^{-1} (Fig. 3B). In this case, the product of the hydratase reaction is L-3,5-dihydroxydecanoyl-CoA (L-3,5-DHD-CoA; see Fig. 4A).

DISCUSSION

Metabolism of 5-HD-CoA

5-Hydroxydecanoate, which blocks preconditioning, is generally presumed to be a selective mitochondrial K_{ATP} channel inhibitor. However, the recent report that 5-HD is thioester linked to CoA via acyl-CoA synthetase (Hanley *et al.* 2002a), producing 5-HD-CoA, undermines this assumption. In the present study, we have now shown that 5-HD-CoA is dehydrogenated by purified MCAD, the first enzyme of the β -oxidation pathway. This result could be predicted from our molecular model of a MCAD-5-HD-CoA complex, which showed that the hydroxyl group on the acyl tail would be unable to sterically hinder the active site. Moreover, we also found that the enoyl-CoA product of this reaction, 5-HD-enoyl-CoA, was a substrate for the next enzyme of the β -oxidation pathway (enoyl-CoA

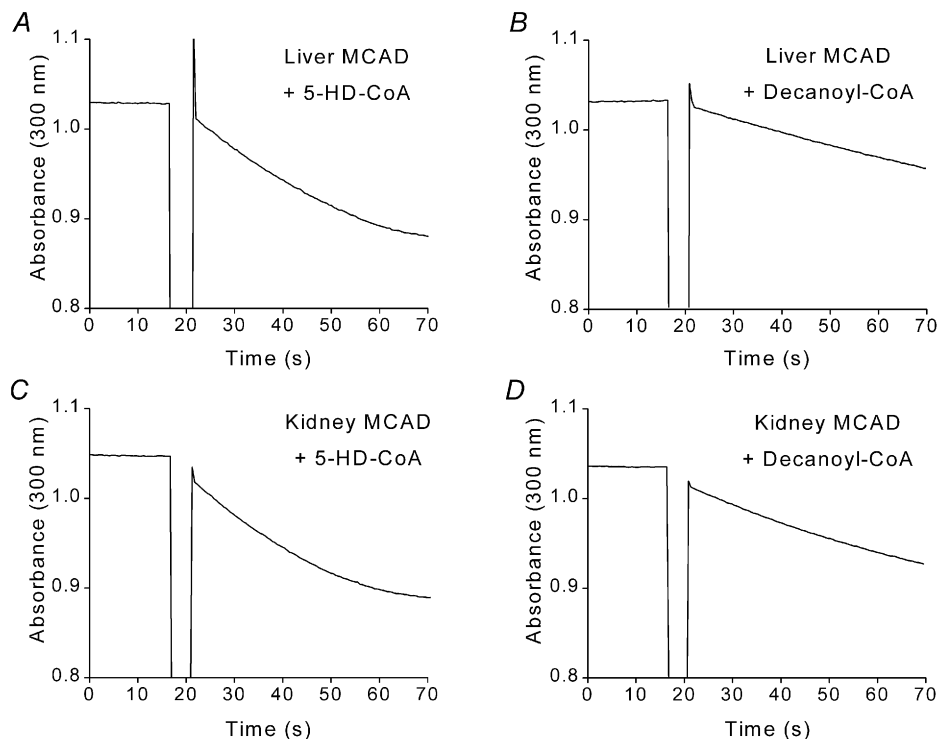


Figure 2. 5-Hydroxydecanoyl-CoA is a substrate for human liver and pig kidney MCAD

A and B, purified human liver MCAD (50 nM) was added (indicated by interruption in trace) to solution containing electron acceptor (FcPF₆) and saturating concentrations ($43 \mu\text{M}$) of either 5-HD-CoA (A) or decanoyl-CoA (B). A decrease in absorbance at 300 nm indicates enzyme activity. C and D, purified pig kidney MCAD (50 nM) was added to solution containing either 5-HD-CoA (C) or decanoyl-CoA (D), each at a concentration of $43 \mu\text{M}$.

hydratase), yielding L-3,5-dihydroxydecanoyl-CoA. The above results are schematically summarised in Fig. 4A.

The K_m of human liver MCAD was about 4-fold higher with 5-HD-CoA as substrate than with decanoyl-CoA as substrate, indicating that 5-HD-CoA binds more weakly to the enzyme. Hence, the hydroxyl group on the acyl tail of 5-HD-CoA evidently decreases the efficiency with which this ligand, compared to decanoyl-CoA, binds to the catalytic pocket of MCAD. However, the V_{max} of MCAD was about 2-fold higher with 5-HD-CoA instead of decanoate as substrate. The most likely explanation for the higher V_{max} is that the weaker binding of 5-HD-CoA facilitates the rate-limiting step of the enzyme, the dissociation of the reaction product (5-HD-enoyl-CoA) from the active site (Peterson *et al.* 1998). Taken together,

the 4-fold higher K_m and 2-fold higher V_{max} of MCAD with 5-HD-CoA as substrate, compared to decanoyl-CoA, suggests that the rate of enzyme turnover would be similar at lower concentrations of either substrate whereas, at higher substrate concentrations, turnover rate would be about 2-fold greater with 5-HD-CoA.

We also showed that purified 5-HD-enoyl-CoA was a substrate for enoyl-CoA hydratase, which suggests that 5-HD may be completely metabolised. Assuming this is the case, and taking into account that 5-HD is a racemic mixture, the last step of β -oxidation, catalysed by β -ketothiolase (EC 2.3.1.16), would yield acetyl-CoA and a racemic mixture of 3-hydroxyoctanoyl-CoA. L-3-Hydroxyoctanoyl-CoA (the L-isomer) is not a metabolic end-product since it is a normal substrate for L-3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), the penultimate enzyme of the β -oxidation pathway. This enzyme is enantioselective and therefore D-3-hydroxyoctanoyl-CoA (the D-isomer) would first need to be converted to L-3-hydroxyoctanoyl-CoA via 3-hydroxyacyl-CoA epimerase before it could be metabolised. However, 3-hydroxyacyl-CoA epimerase activity has been shown to

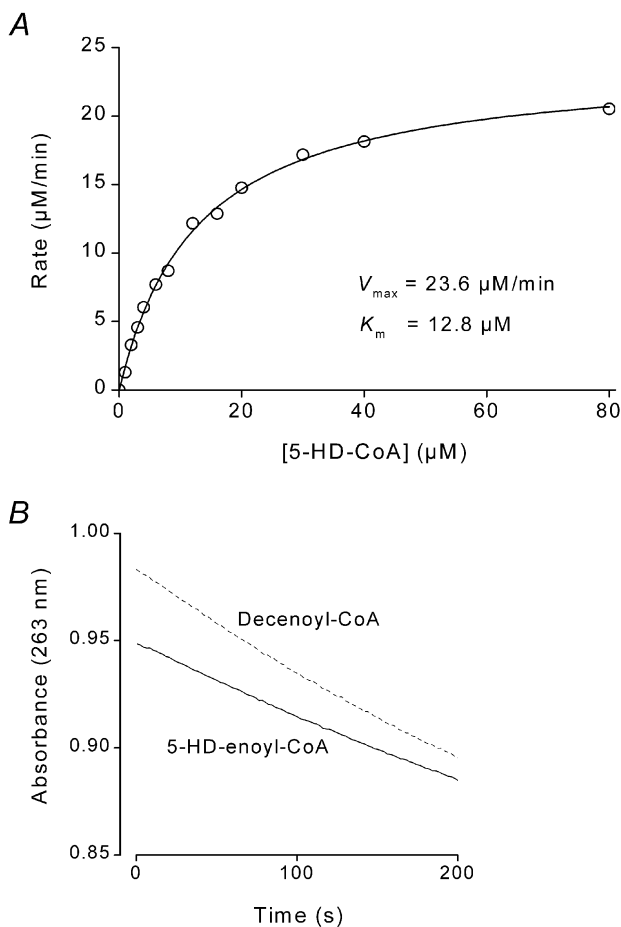


Figure 3. Kinetics of MCAD-catalysed dehydrogenation of 5-HD-CoA and metabolism of the product 5-hydroxydecanoyl-CoA

A, steady-state activity of human liver MCAD was determined at various concentrations of purified 5-HD-CoA. K_m was $12.8 \pm 0.6 \mu\text{M}$ and V_{max} $23.6 \mu\text{M min}^{-1}$. Data were fitted using the Michaelis-Menten equation (see Results). *B*, bovine liver enoyl-CoA hydratase was activated by addition of either decenoyl-CoA (dashed line) or purified 5-hydroxydecanoyl-CoA (continuous line). Enzyme activity was monitored by measuring absorbance at 263 nm.

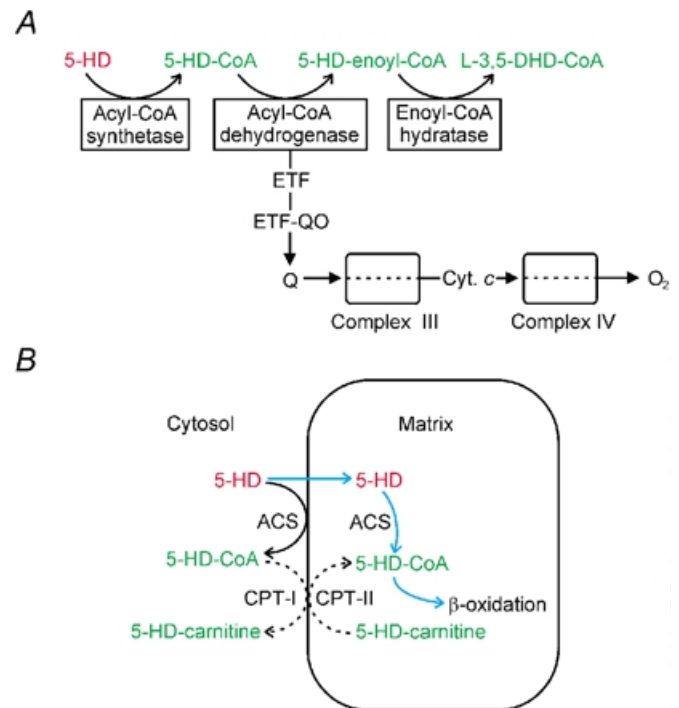


Figure 4. Schematic diagrams of the metabolism of 5-HD and its uptake into the mitochondrial matrix

A, schematic summary showing activation and β -oxidation of 5-hydroxydecanoate. Medium-chain acyl-CoA dehydrogenase transfers electrons to the electron transport chain via electron transfer flavoprotein (ETF) and ETF oxidoreductase (ETF-QO). *B*, schematic diagram showing that, typical for medium-chain fatty acids, 5-HD can be activated via ACS (acyl-CoA synthetase) either on the mitochondrial outer membrane or in the matrix. Whether extramitochondrial 5-HD-CoA serves as a substrate for CPT-I (carnitine palmitoyltransferase-I) is not known.

be weak in heart mitochondria (Chu *et al.* 1984). If D-3-hydroxyoctanoyl-CoA is not degraded by β -oxidation, the acyl group would probably exit the mitochondria as the free acid (after thioesterase-catalysed hydrolysis) or as the carnitine derivative, after transfer to carnitine via carnitine acyltransferase.

Implications for preconditioning

Despite extensive study, the mechanisms underlying the protection of the heart against ischaemic damage have remained unclear. There are three main mechanisms that have been proposed to explain the cardioprotection conferred by drugs (pharmacological preconditioning) or by brief episodes of ischaemia (ischaemic preconditioning) (Gross & Fryer, 1999; Grover & Garlid, 2000; Toyoda *et al.* 2000; Sanada *et al.* 2001; Suzuki *et al.* 2002; Hanley *et al.* 2002a, b; Standen, 2002): (i) activation of sarcolemmal K_{ATP} channels, (ii) activation of mitochondrial K_{ATP} channels, and (iii) inhibition of components of the respiratory chain. Since 5-HD has been shown to inhibit both ischaemic and pharmacological preconditioning (Gross & Fryer, 1999; Gross, 2000; Sato & Marbán, 2000) elucidation of its molecular action(s) may provide a clue to the mechanism of preconditioning.

The data reported here, together with the results of Hanley *et al.* (2002a), extend the possible mechanisms by which 5-HD could block preconditioning: (i) the putative mitochondrial K_{ATP} channel may be inhibited by cytosolic 5-HD or 5-HD-CoA; (ii) intermediates of 5-HD metabolism may inhibit the β -oxidation pathway or exert some other cellular action; (iii) 5-HD may be completely metabolised via the β -oxidation pathway, which is linked to the respiratory chain. This link may provide a bypass to respiratory chain inhibition at complex I or complex II (Hanley *et al.* 2002a). β -Oxidation of 5-HD would also explain the observation that 5-HD reverses the opening of sarcolemmal K_{ATP} channels induced by blocking glucose metabolism in ventricular myocytes (Notsu *et al.* 1992). (iv) Application of 5-HD may lead to a switch of metabolism from glucose to fatty acid oxidation, which may impair recovery of cardiac function after ischaemia (Lopaschuk, 1997).

Like other short- or medium-chain fatty acids, 5-HD can traverse the mitochondrial inner membrane. Subsequently, 5-HD can be activated by medium-chain acyl-CoA synthetase present in the matrix, as illustrated in Fig. 4B (blue arrows), and metabolised by β -oxidation (Fig. 4A). 5-HD-CoA produced by acyl-CoA synthetase at the mitochondrial outer membrane, however, is membrane impermeant and needs to access the matrix via carnitine palmitoyltransferase-I (CPT-I) before it can be metabolised. Whether extramitochondrially activated 5-HD serves as a substrate for CPT-I is not known (Fig. 4B, interrupted lines), although this is likely the case.

In conclusion, our results suggest that metabolism of 5-HD may be responsible for its inhibitory effect on preconditioning. Thus, 5-HD should no longer be considered a suitable pharmacological tool for the identification of mitochondrial ATP-sensitive potassium channels.

REFERENCES

- Chu CH, Kushner L, Cuebas D & Schulz H (1984). The activity of 3-hydroxyacyl-CoA epimerase is insufficient to account for the rate of linoleate oxidation in rat heart mitochondria. Evidence for a modified pathway of linoleate degradation. *Biochem Biophys Res Commun* **118**, 162–167.
- Cope DK, Impastato WK, Cohen MV & Downey JM (1997). Volatile anesthetics protect the ischemic rabbit myocardium from infarction. *Anesthesiology* **86**, 699–709.
- Garlid KD, Paucek P, Yarov-Yarovoy V, Murray HN, Darbenzio RB, D'Alonzo AJ, Lodge NJ, Smith MA & Grover GJ (1997). Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K^+ channels. Possible mechanism of cardioprotection. *Circ Res* **81**, 1072–1082.
- Gross GJ (2000). The role of mitochondrial K_{ATP} channels in cardioprotection. *Basic Res Cardiol* **95**, 280–284.
- Gross GJ & Fryer RM (1999). Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. *Circ Res* **84**, 973–979.
- Grover GJ & Garlid KD (2000). ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. *J Mol Cell Cardiol* **32**, 677–695.
- Hanley PJ, Mickel M, Löffler M, Brandt U & Daut J (2002a). K_{ATP} channel-independent targets of diazoxide and 5-hydroxydecanoate in the heart. *J Physiol* **542**, 735–741.
- Hanley PJ, Ray J, Brandt U & Daut J (2002b). Halothane, isoflurane and sevoflurane inhibit NADH:ubiquinone oxidoreductase (complex I) of cardiac mitochondria. *J Physiol* **544**, 687–693.
- Jabůrek M, Yarov-Yarovoy V, Paucek P & Garlid KD (1998). State-dependent inhibition of the mitochondrial K_{ATP} channel by glyburide and 5-hydroxydecanoate. *J Biol Chem* **273**, 13578–13582.
- Johnson JK, Wang ZX & Srivastava DK (1992). Mechanistic investigation of medium-chain fatty acyl-CoA dehydrogenase utilizing 3-indolepropionyl/acryloyl-CoA as chromophoric substrate analogues. *Biochemistry* **31**, 10564–10575.
- Kim J-JP, Wang M & Paschke R (1993). Crystal structures of medium-chain acyl-CoA dehydrogenase from pig liver mitochondria with and without substrate. *Proc Natl Acad Sci U S A* **90**, 7523–7527.
- Knudsen J, Jensen MV, Hansen JK, Fæbergeman NJ, Neergaard TBF & Gaigg B (1999). Role of acylCoA binding protein in acylCoA transport, metabolism and cell signaling. *Mol Cell Biochem* **192**, 95–103.
- Kumar NR & Srivastava DK (1994). Reductive half-reaction of medium-chain fatty acyl-CoA dehydrogenase utilizing octanoyl-CoA/octenoyl-CoA as a physiological substrate/product pair: similarity in the microscopic pathways of octanoyl-CoA oxidation and octenoyl-CoA binding. *Biochemistry* **33**, 8833–8841.
- Lee HJ, Wang M, Paschke R, Nandy A, Ghisla S & Kim JJ (1996). Crystal structures of the wild type and the Glu376Gly/Thr255Glu mutant of human medium-chain acyl-CoA dehydrogenase: influence of the location of the catalytic base on substrate specificity. *Biochemistry* **35**, 12412–12420.

- Liu GX, Hanley PJ, Ray J & Daut J (2001). Long-chain acyl-coenzyme A esters and fatty acids directly link metabolism to K_{ATP} channels in the heart. *Circ Res* **88**, 918–924.
- Lopaschuk GD (1997). Alterations in fatty acid oxidation during reperfusion of the heart after myocardial ischemia. *Am J Cardiol* **80**(3A), 11A–16A.
- Notsu T, Tanaka I, Takano M & Noma A (1992). Blockade of the ATP-sensitive K^+ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. *J Pharmacol Exp Ther* **260**, 702–708.
- Paucek P, Yarov-Yarovoy V, Sun X & Garlid KD (1996). Inhibition of the mitochondrial K_{ATP} channel by long-chain acyl-CoA esters and activation by guanine nucleotides. *J Biol Chem* **271**, 32084–32088.
- Peterson KL, Galitz DS & Srivastava DK (1998). Influence of excision of a methylene group from Glu-376 (Glu376 Asp mutation) in the medium chain acyl-CoA dehydrogenase-catalyzed reaction. *Biochemistry* **37**, 1697–1705.
- Peterson KL, Sergienko EE, Wu Y, Kumar NR, Strauss AW, Oleson AE, Muhonen WW, Shabb JB & Srivastava DK (1995). Recombinant human liver medium-chain acyl-CoA dehydrogenase: purification, characterization, and the mechanism of interactions with functionally diverse C_8 -CoA molecules. *Biochemistry* **34**, 14942–14953.
- Peterson KM, Gopalan KV, Nandy A & Srivastava DK (2001). Influence of Glu-376 Gln mutation on enthalpy and heat capacity changes for the binding of slightly altered ligands to medium chain acyl-CoA dehydrogenase. *Protein Sci* **10**, 1822–1834.
- Roberts DL, Frerman FE & Kim J-JP (1996). Three-dimensional structure of human electron transfer flavoprotein to 2.1-Å resolution. *Proc Natl Acad Sci U S A* **93**, 14355–14360.
- Sanada S, Kitakaze M, Asanuma H, Harada K, Ogita H, Node K, Takashima S, Sakata Y, Asakura M, Shinozaki Y, Mori H, Kuzuya T & Hori M (2001). Role of mitochondrial and sarcolemmal K_{ATP} channels in ischemic preconditioning of the canine heart. *Am J Physiol Heart Circ Physiol* **280**, H256–263.
- Sato T & Marbán E (2000). The role of mitochondrial K_{ATP} channels in cardioprotection. *Basic Res Cardiol* **95**, 285–289.
- Schulz R, Cohen MV, Behrends M, Downey JM & Heusch G (2001). Signal transduction of ischemic preconditioning. *Cardiovasc Res* **52**, 181–198.
- Standen NB (2002). Cardioprotection by preconditioning: K_{ATP} channels, metabolism, or both? *J Physiol* **542**, 666.
- Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Seino S, Marban E & Nakaya H (2002). Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest* **109**, 509–516.
- Thorpe C & Kim J-JP (1995). Structure and mechanism of action of the acyl-CoA dehydrogenases. *FASEB J* **9**, 718–725.
- Toyoda Y, Friehs I, Parker RA, Levitsky S & McCully JD (2000). Differential role of sarcolemmal and mitochondrial K_{ATP} channels in adenosine-enhanced ischemic preconditioning. *Am J Physiol Heart Circ Physiol* **279**, H2694–2703.
- Waterson RM & Hill RL (1972). Enoyl coenzyme A hydratase (crotonase). Catalytic properties of crotonase and its possible regulatory role in fatty acid oxidation. *J Biol Chem* **247**, 5258–5265.

Acknowledgements

We thank Professor Horst Schulz (Department of Chemistry, City University of New York) for helpful discussion.