EFFECTS OF PENT-4-ENOATE ON FLUX THROUGH ACYL-CoA DEHYDROGENASES OF β-OXIDATION IN INTACT RAT LIVER MITOCHONDRIA

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1. Introduction

The inhibitory action of the hypoglyceamic pent-4-enoic acid on mitochondrial metabolism has been studied with isolated mitochondria [1,2], as well as perfused liver [3] and heart [4]. These studies have not, however, given results which are conclusive as to the mechanism of action of pent-4-enoic acid, particularly as regards its inhibitory effect on β-oxidation of other fatty acids. It is generally agreed that pent-4-enoic acid is well metabolised by perfused organs at perfusate concentrations less than 1–2 mM, its inhibitory properties becoming manifested at higher concentrations. With isolated mitochondria it is also possible to demonstrate persistant oxidation of pent-4-enoate present at a low concentration, provided that a high concentration of L-carnitine has been included [5]. Again higher concentrations of pent-4-enoate (>300 μM) inhibits its own oxidation [5]. With pent-4-enoylcarnitine, however, it has not been possible to demonstrate persistant oxidation of this fatty acid [2]. The reason for this may involve different rates of acylation of mitochondrial CoA [6].

The inhibition of β-oxidation of other fatty acids by pent-4-enoate requires its conversion to pent-4-enoyl-CoA [2]. It has been suggested that the inhibitory effect of pent-4-enoate is simply due to sequestration of cellular CoA and carnitine as unmetabolisable esters [7]. It has been shown, however, that the inhibition of β-oxidation can be dissociated from the inhibition of other mitochondrial reaction requiring CoA, e.g., those involved in the oxidation of pyruvate [1,5]. This finding strongly suggests that the inhibitory action of pent-4-enoate is not purely a matter of CoA sequestration, although this may be involved in some aspects of its action [6]. The sensitivity of β-oxidation to low concentrations of pent-4-enoate (and the absence of inhibition of pyruvate oxidation under identical conditions) suggests that a pent-4-enoate metabolite must have a powerfully inhibitory action somewhere in the β-oxidation sequence. The apparently irreversible inhibitory effect of penta-2,4-dienoyl-CoA on acetoacetyl-CoA thiolase (EC 2.3.1.9) observed with the purified enzyme [8] and with soluble mitochondrial extracts [5], is not found following exposure of intact mitochondria to pent-4-enoate [5]. In intact mitochondria the inhibitory effect of pent-4-enoate is therefore reversible, and the inhibitory site may be located prior to acetoacetyl-CoA thiolase in the β-oxidation system.

Recently an assay which selectively measures the acyl-CoA flux through the acyl-CoA dehydrogenases (EC 1.3.99.3) has been developed [9]. These enzymes may represent the rate-limiting stage of β-oxidation [10], and it is therefore of some interest to examine effects of pent-4-enoylcarnitine with this assay.

2. Materials and methods

Liver mitochondria from male albino Wistar rats (150–200 g) were prepared as in [9]. Acylcarnitines were prepared as in [11]. The purity of the preparations were checked with thin-layer chromatography [11] and by gas chromatography, the free acid content was less than 1%.

The acylcarnitine-dependent reduction of ferri-

cyanide was measured by using an Aminco DW-2 spectrophotometer, operated in dual wavelength mode at 420 nm and 475 nm. The details of the assay are in [9]. With this assay the rate of reduction of ferricyanide is a direct measure of the acyl-CoA flux through the acyl-CoA dehydrogenases. Maximal rates of β-oxidation by rotenone-blocked mitochondria were achieved by the addition of 10 mM oxaloacetate, the latter being acceptor for both reducing equivalents as well as acetyl-groups (see [9]).

Purified hypoglycin was a gift from Dr H. S. A. Sherratt, Dept. Pharmacol. Sci. University of Newcastle upon Tyne, and had been prepared as in [5].

Mitochondrial protein concentrations were assayed by using the biuret assay [12], with Precimat (Boehringer GMBH, Mannheim) protein standards.

3. Results and discussion

When the mitochondrial oxidation of saturated or unsaturated acylcarnitines is measured by using the spectrophotometric assay [9] traces qualitatively identical to that shown in fig.1 with myristoylcarnitine are obtained, viz., the addition of acylcarnitine give some stimulation of the rate of reduction of ferricyanide. This represents the rotenone-insensitive component of β-oxidation [9,13]. This rate is stimulated several-fold on the addition of 10 mM oxaloacetate (see fig.1(c)). The addition of pent-4-enoylcarnitine similarly give a rotenone-insensitive rate of β-oxidation. On the addition of oxaloacetate, however, this rate is instantaneously blocked, as is also the oxidation of subsequently added myristoylcarnitine. On adding oxaloacetate prior to the addition of pent-4-enoylcarnitine there is no apparent oxidation of this acylcarnitine. Surprisingly, the oxidation of subsequently added myristoylcarnitine is also now completely blocked (see fig.1).

The results presented in fig.2 show that the rotenone-insensitive rate of oxidation of pent-4-enoylcarnitine decreases towards zero with time (in the absence of added oxaloacetate). This is analogous to the self-limiting pulse of respiration with pent-4-enoylcarnitine [2]. The oxidation of myristoylcarnitine added towards the end of the pulse of pent-4-enoylcarnitine oxidation is also 100% inhibited, also in agreement with earlier polarographic results

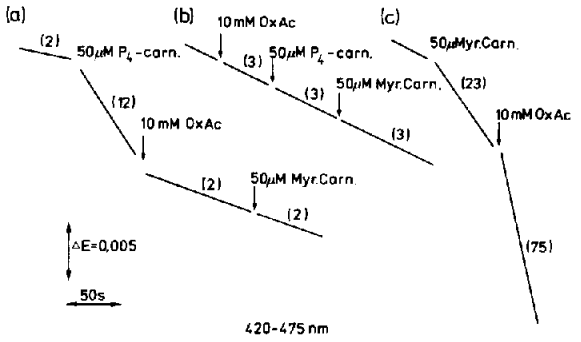

Fig.1. The effect of oxaloacetate on the pent-4-enoyl-carnitine dependent flux through acyl-CoA dehydrogenases. The reduction of ferricyanide was recorded as described in section 2. Typical traces obtained when 10 mM oxaloacetate is added after pent-4-enoylcarnitine (a) and before pent-4-enoylcarnitine (b). A trace obtained with myristoylcarnitines, which is qualitatively identical to traces obtained with all other saturated acylcarnitines tested is also shown (c). In this case the order of addition of oxaloacetate is of no significance to the final rate (not shown). The numbers in parentheses represent rates of ferricyanide reduction expressed as nmol/min/mg protein.
Abbreviations: OxAc, oxaloacetate; P_4-carn, pent-4-enoyl-carnitine; Myr.carn, myristoylcarnitine. Each assay contained 1.6 mg mitochondrial protein.

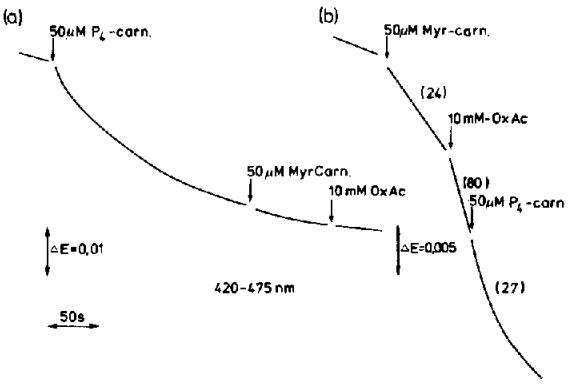

Fig.2. The self-limiting nature of rotenone-insensitive pent-4-enoylcarnitine oxidation. A typical trace, obtained as in section 2, showing that pent-4-enoylcarnitine dependent acyl-CoA dehydrogenase flux decreases with time (a). A trace showing the effect of addition of pent-4-enoylcarnitine during sustained oxidation of myristoylcarnitine is also shown (b). Each assay contained 2.3 mg mitochondrial protein. The assay details are otherwise as in fig.1 legend.

[2]. Results presented in fig.2 also show that when pent-4-enoyl-carnitine is added during sustained oxidation of myristoylcarnitine, progressive inhibition of the acyl-CoA flux results.

The hypoglycin metabolite methylenecyclopropylacetyl-CoA selectively inhibits butyryl-CoA dehydrogenase (EC 1.3.99.2), while palmitoyl-CoA dehydrogenase is not inhibited [14,15]. It was therefore of interest to study the effect of blocked butyryl-CoA dehydrogenase on pent-4-enoylcarnitine-dependent acyl-CoA fluxes. Rats which had been deprived of food for 12 h were given 80 mg hypoglycin/kg body wt. Control rats were given a similar volume of saline. After further 12 h livers were removed, and mitochondria prepared. Some aspects of the effect of hypoglycin administration on acyl-CoA dehydrogenase flux are in [9]. As expected the oxidation of butyrylcarnitine is completely blocked, while there is some residual oxidation of subsequently added hexanoylcarnitine (see fig.3). Interestingly, hexanoylcarnitine oxidation by mitchondria from hypoglycin-treated rats shows a property characteristic of butyrylcarnitine oxidation by control mitochondria, in that its oxidation is not further stimulated by added oxaloacetate (fig.3). Myristoylcarnitine gives traces which are qualitatively identical to those of control mitochondria, although the post-oxaloacetate rate of oxidation is only about 50% of the control rate. This agrees with [9,14]. Results presented in fig.3 also show that the rotenone-insensitive oxidation of pent-4-enoylcarnitine is completely abolished in mitochondria from hypoglycin treated rat, the addition of oxaloacetate being without any detectable effect.

This investigation shows that pent-4-enoylcarnitine is treated by the β-oxidation system in a manner not shown by any other unsaturated acylcarnitines which has been investigated (H. O. unpublished). The inhibitory effect of oxaloacetate strongly suggests that some metabolite of pent-4-enoyl-CoA is powerfully inhibitory to β-oxidation. It is likely that an inhibitory concentration of this metabolite is formed practically instantaneously when the NADH-block of β-oxidation (imposed by rotenone) is bypassed on the addition of oxaloacetate.

A very small amount of this metabolite, perhaps 3-oxo-pent-4-enoyl-CoA, is clearly adequate to cause nearly 100% block of the oxidation of subsequently

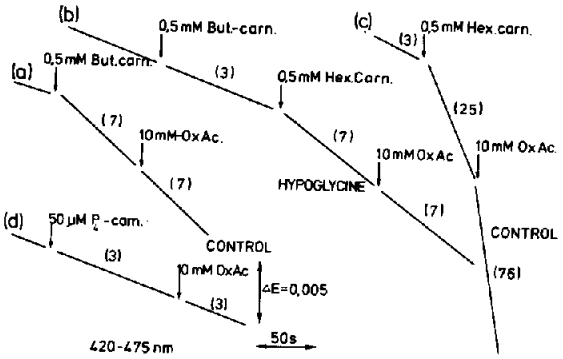

Fig.3. The effect of hypoglycine-induced block of butyryl-CoA dehydrogenase on acylcarnitine dependent fluxes through the acyl-CoA dehydrogenases. Typical traces obtained from butyrylcarnitine (a) and hexanoylcarnitine (b) with control mitochondria (1.1 mg protein/assay), and with mitochondria from a hypoglycine-treated rat (c) (1.4 mg mitochondrial protein/assay). The effect of hypoglycine treatment of oxidation of pent-4-enoylcarnitine is also shown (d).

Abbreviations: But.carn, butyrylcarnitine; Hex.carn, hexanoyl-carnitine.

The details of the assay are otherwise as in fig.1 legend.

added myristoylcarnitine. This is indicated by the inhibited myristoylcarnitine oxidation also observed when oxaloacetate had been added to the mitochondria prior to the addition of pent-4-enoylcarnitine (see fig.1). In this case there was no detectable oxidation of pent-4-enoylcarnitine. The cause of this inhibition cannot simply be due to CoA sequestration as unmetabolised pent-4-enoyl-CoA (pent-4-enoyl-carnitine is an excellent substrate for carnitine acetyl-transferase (EC 2.3.1.7) [18]), as is shown by the oxidation of pent-4-enoylcarnitine which takes place in the absence of added oxaloacetate (fig.2). The amount of ferricyanide reduced during this self-limiting pulse of oxidation corresponds to the passage of about 15 nmoles pent-4-enoyl-CoA through the acyl-CoA dehydrogenase. This corresponds to at least 3 times available intra-mitochondrial CoA, assuming a value of about 2 nmol CoA/mg mitochondrial protein [2,16]. Therefore recycling of CoA must have taken place before β-oxidation ceases. Similar results were reported on the basis of polarographic work [2]. It is therefore likely that the rotenone-insensitive oxidation of pent-4-enoylcarnitine is self-limiting because the

build up of an inhibitory concentration of the required metabolite is very slow under these conditions. This also suggests that the inhibitory metabolite is generated by a reaction distally in the β-oxidation sequence to the NADH-block, i.e., by the β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) or by the acetoacetyl-CoA thiolase. The enoyl-CoA hydratase (EC 4.2.1.17) does not appear to be inhibited in rotenone-blocked mitochondria as β-hydroxyacyl-esters have been found to accumulate under these conditions [13].

The absence of rotenone-insensitive oxidation of pent-4-enoylcarnitine in mitochondria blocked with butyryl-CoA dehydrogenases show that the oxidation of this acyl-CoA species is solely due to the activity of butyryl-CoA dehydrogenase. It is therefore noteworthy that an acyl-CoA species which is metabolised by butyryl-CoA dehydrogenase is able to inhibit the oxidation of fatty acids of all chain lengths [2].

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