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ON THE INHIBITION OF α-OXOBUTYRATE UTILIZATION BY FATTY ACIDS IN RAT LIVER MITOCHONDRIA

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SUMMARY

It has been found that free fatty acids and acylcarnitine inhibit α-oxobutyrate utilization in rat liver mitochondria. It has been recognized that the intramitochondrial accumulation of acetyl-CoA, produced by the β-oxidation of activated fatty acids, is responsible for such inhibition. In fact acetyl-CoA is shown to inhibit α-oxobutyrate dehydrogenase (α-oxoglutarate:lipoate oxidoreductase (acceptor acylating) EC 1.2.4.2)

INTRODUCTION

It has been shown that α-oxobutyrate inhibits the oxidation of fatty acids, thus preventing the formation and accumulation of ketone bodies[1]. This action has been attributed to a competition between α-oxobutyrate and fatty acid for the utilization of intramitochondrially available ATP and CoA[1]. Fatty acids and α-oxobutyrate require CoA for the formation of acetyl-CoA and propionyl-CoA, respectively. Furthermore, the activation of fatty acids requires ATP as does the carboxylation step in the utilization of α-oxobutyrate leading from propionyl-CoA to methylmalonyl-CoA.

It was reasonable to suppose that fatty acids in turn could influence the mitochondrial utilization of α-oxobutyrate in some way. Evidence that fatty acids inhibit α-oxobutyrate utilization through the formation of acetyl-CoA, which is an inhibitor of α-oxobutyrate dehydrogenase (α-oxoglutyrate:lipoate oxidoreductase (acceptor acylating), EC 1.2.4.2) is presented in this paper.

METHODS AND MATERIALS

Mitochondria were isolated from liver of Wistar albino rats by the conventional 0.25 M sucrose procedure.

α-Oxobutyrate and pyruvate were determined enzymatically according to BÜCHER *et al.*[2].

The activity of partially purified pyruvate dehydrogenase (pyruvate:lipoate oxidoreductase (acceptor acylating), EC 1.2.4.1), prepared according to SCRIBA AND HOLZER[3], was measured by the method of GARLAND AND RANDLE[4]. The assay

is based on the two-stage oxidation of α-hydroxybutyrate to α-oxobutyrate and to propionyl-CoA, which yields 2 moles of NADH per mole of propionyl-CoA formed. NADH formation was followed spectrophotometrically at 340 nm.

$H^{14}CO_3^-$ incorporation was determined in the following way: 0.3 ml of 5 M HCl was added to 1.0 ml of the incubation mixture; after centrifugation the mitochondrial proteins were discarded and 1.0 ml of the supernatant was pipetted into the counting vial and stirred for 20 min at 40° in order to eliminate residual $H^{14}O_3^-$; after cooling, 10 ml of the scintillation liquid were added to the sample and radioactivity was measured in a Packard Tricarb spectrometer.

Acyl-CoA was determined according to SÖLING *et al.*[5]. Mitochondrial proteins were determined by the usual biuret method[6].

All reagents were of analytical grade.

Sodium α-oxobutyrate was purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). $NaH^{14}CO_3$ was obtained from the Radiochemical Centre, Amersham (England).

RESULTS

Table I shows that octanoate and palmitoylcarnitine significantly prevented the disappearance of α-oxobutyrate and the incorporation of $HC^{14}O_3^-$. The fact that α-oxobutyrate disappearance and $HC^{14}O_3^-$ incorporation were parallel indicates that α-oxobutyrate was transformed up to, or beyond, the carboxylation step leading to the formation of methylmalonyl-CoA.

TABLE I

ACTION OF FATTY ACIDS ON α-OXOBUTYRATE DISAPPEARANCE, $H^{14}CO_3^-$ INCORPORATION AND ACETYL-CoA CONCENTRATION IN RAT LIVER MITOCHONDRIA

The incubation mixture contained in a final volume of 2 ml: 10 mM phosphate buffer (pH 7.4), 18 mM NaCl, 40 mM KCl, 4 mM $MgCl_2$, 25 mM sucrose, 0.05 mM NAD^+, 5 mM α-oxobutyrate, 5 mM $NaH^{14}CO_3$ (specific activity $0.8 \cdot 10^2$ counts/min per nmole), 0.05 mg oligomycin, 15 mg mitochondrial proteins. Temperature, 37°. Time, 5 min.

Additions	*α-Oxobutyrate disappearance (nmoles/mg protein per min)*	*$H^{14}CO_3^-$ incorporation (nmoles/mg protein per min)*	*Acetyl-CoA concn. (nmoles/10 mg protein)*
(1) 3 mM ATP	20	6.45	0.10
(2) 3 mM ATP, 1 mM octanoate	8	2.77	1.50
(3) 3 mM ATP, 0.2 mM palmitoylcarnitine	12	3.10	1.20
(4) None	15	0	0.05
(5) 1 mM octanoate	15	0	0.05
(6) 0.2 mM palmitoylcarnitine	8	0	0.98

It can also be observed that in the absence of added ATP no incorporation of $H^{14}CO_3^-$ occurs, while a significant amount of α-oxobutyrate still disappeared. It can be deduced that in the absence of ATP, α-oxobutyrate utilization is limited to the formation of propionyl-CoA, *i.e.* to a step preceeding the carboxylation reaction which is ATP dependent. It is expected that under these conditions propionyl-CoA accumulates.

Furthermore, in the absence of ATP, palmitoylcarnitine still inhibited α-oxobutyrate disappearance, while octanoate was ineffective. This indicates that in order to inhibit α-oxobutyrate utilization fatty acids must be activated and presumably oxidized.

In fact, as is shown in Fig. 1, the ability of fatty acids to inhibit oxobutyrate utilization which is proportional to their carbon chain length strictly paralleled their known rate of activation and oxidation[7].

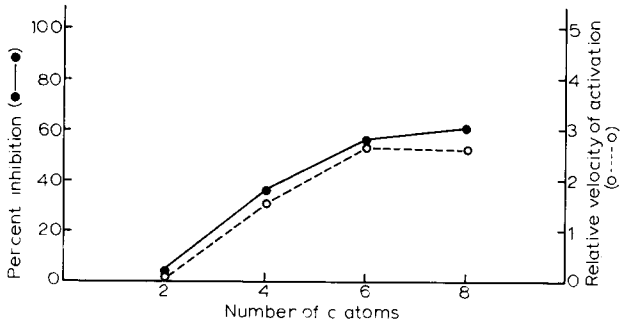

Fig. 1. Comparison of percent inhibition of α-oxobutyrate oxidation by different fatty acids and their relative activation velocity. The inhibition was determined by measuring the incorporation of $H^{14}CO_3^-$ into α-oxobutyrate under conditions described in Table I, Expt. 2. Fatty acids were added at the following concentrations: 4 mM acetate, 2 mM butyrate, 2 mM hexanoate and 1 mM octanoate. The relative activation velocity of the fatty acids employed is taken from MAHLER *et al.*[7].

As it is known, fatty acid activation involves a utilization of ATP and CoA and both these factors are also necessary for the transformation of α-oxobutyrate to methylmalonyl-CoA. The possibility that fatty acid could have inhibited α-oxobutyrate disappearance and $H^{14}CO_3^-$ incorporation by inducing an intramitochondrial shortage of ATP can be ruled out in the light of the results reported in Table II.

These results show that in the presence of NAD^+, oligomycin and ATP, octanoate inhibited the incorporation of $H^{14}CO_3^-$ in the presence of α-oxobutyrate, but stimulated the $H^{14}CO_3^-$ incorporation in the presence of pyruvate.

TABLE II

ACTION OF OCTANOATE ON α-OXOBUTYRATE AND PYRUVATE DISAPPEARANCE, AND ON $H^{14}CO_3^-$ INCORPORATION IN THE PRESENCE OF α-OXOBUTYRATE AND PYRUVATE

Incubation medium as in Table I, without α-oxobutyrate, *plus* 3 mM ATP. Temperature and time as in Table I.

Additions	*Substrate disappearance (nmoles/mg protein per min)*	*$H^{14}CO_3^-$ incorporation (nmoles/mg protein per min)*
(1) 5 mM α-oxobutyrate	20	6.45
(2) 5 mM α-oxobutyrate, 1 mM octanoate	8	2.77
(3) 5 mM pyruvate	18	15.1
(4) 5 mM pyruvate, 1 mM octanoate	7.5	25.0

Since the carboxylation of pyruvate to oxaloacetate and of propionyl-CoA to methylmalonyl-CoA are both ATP dependent, a shortage of ATP does not appear to be the cause of the observed effects of octanoate.

Another possibility was that α-oxobutyrate inhibition was due to the accumulation of acetyl-CoA produced during fatty acid oxidation.

It is known that acetyl-CoA inhibits pyruvate dehydrogenase[4], which is considered responsible also for the oxidative decarboxylation of α-oxobutyrate[8], the upper homologue of pyruvate.

Indeed as is shown in Fig. 2 acetyl-CoA added to a partially purified preparation of pyruvate dehydrogenase inhibits the oxidative decarboxylation of α-oxobutyrate. This inhibition is of the same order of that observed using pyruvate as substrate[1].

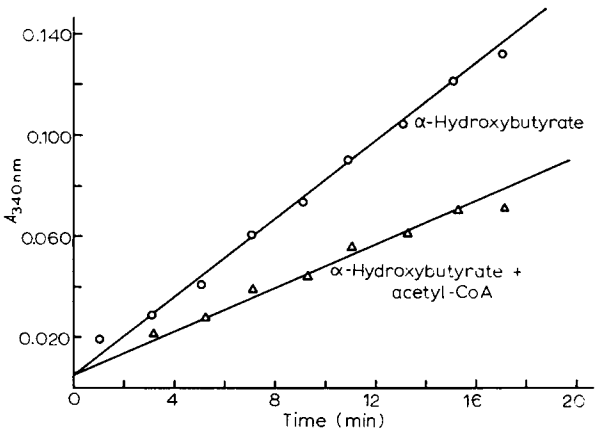

Fig. 2. Action of acetyl-CoA on α-oxobutyrate oxidation by a partially purified preparation of pyruvate dehydrogenase. In a final volume of 1.8 ml the mixture contained: 130 mM Tris–HCl buffer (pH 7.4), 15 mM $MgCl_2$, 2.5 mM EDTA, 1 mM thiamine pyrophosphate, 1 mM NAD^+, 5 mM 2-mercaptoethanol, 10 units of lactate dehydrogenase (Boehringer), 0.01 unit of pyruvate dehydrogenase, 10 mM α-hydroxybutyrate. Where indicated 500 nmoles of acetyl-CoA were added. The reaction was started by addition of 500 nmoles of CoA. As previously mentioned (see METHODS AND MATERIALS), α-hydroxybutyrate is used in the place of α-oxobutyrate for obvious technical reasons.

That acetyl-CoA really accumulates when mitochondria are incubated in the presence of fatty acid and ATP, or palmitoylcarnitine in the absence of ATP, is demonstrated by the results reported in the last column of Table I.

It is likely that the acetyl-CoA concentration reached within the mitochondria when fatty acids are oxidized under the conditions described, is sufficient to inhibit oxo-acid dehydrogenase.

DISCUSSION

The results reported in the present paper clearly show that fatty acid oxidation, through the formation of acetyl-CoA, inhibits α-oxobutyrate utilization by rat liver mitochondria.

This conclusion has been drawn from the evidence that acetyl-CoA inhibits

α-oxobyutrate dehydrogenase (see Fig. 2) and that an accumulation of acetyl-CoA occurs within liver mitochondria incubated in the presence of fatty acid or acylcarnitine (see Table I).

Even though the inhibition of the purified enzyme is lower than that observed with isolated mitochondria, it is tempting to speculate that within the mitochondria the conditions are such that the acetyl-CoA inhibition on α-oxobutyrate dehydrogenase might be more pronounced.

The observation that $H^{14}CO_3^-$ incorporation in mitochondria incubated in the presence of α-oxobutyrate is inhibited by fatty acid or acylcarnitine, lends further support to our conclusion. Such an inhibition is the consequence of a reduced oxidation to propionyl-CoA, the step preceeding the carboxylation reaction leading to methylmalonyl-CoA. On the contrary, fatty acid and acylcarnitine have been found to stimulate $H^{14}CO_3^-$ incorporation in the presence of pyruvate (see Table II). The latter observation is in line with the knowledge that acetyl-CoA stimulates pyruvate carboxylase[9].

From the present results it appears that in mitochondria α-oxobutyrate oxidative decarboxylation is controlled by the level of intramitochondrial acetyl-CoA, while the following carboxylation step, leading to methylmalonyl-CoA is controlled by ATP.

BØHMER AND BREMER[10] have shown that under different physiological conditions the acetylcarnitine content increases when propionylcarnitine decreases and *vice versa*.

Assuming that propionyl-CoA and propionylcarnitine[10], as well as acetyl-CoA and acetylcarnitine are in a close equilibrium[11], the present results, together with those related to the mechanism by which α-oxobutyrate and propionate inhibit fatty acid oxidation[1], give a reasonable explanation for BØHMER AND BREMER's[10] observations.

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