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# INTERACTION BETWEEN OXIDATIVE DECARBOXYLATION OF BRANCHED CHAIN α-KETO ACIDS AND OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

Hans Schweiger and Karl Brand

Institute for Physiological Chemistry, University of Erlangen-Nuremberg
Fahrstr. 17. D-8520 Erlangen, FRG.

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Oxidation of [1-$^{14}$C] -α-ketoisocaproate to yield $^{14}CO_2$ has been studied in respiring rat liver mitochondria. So long as oxidative phosphorylation proceeds the rate of decarboxylation is low. When ATP formation from added ADP is completed the rate of oxidative decarboxylation of α-ketoisocaproate increases abruptly. These observations indicate an inverse correlation between branched chain α-keto acid oxidation and oxidative phosphorylation in liver mitochondria.

There are several reports on dietary (1,2) and hormonal (1) regulation of branched chain α-keto acid dehydrogenase (EC 1.2.1.25) in liver. This enzyme is involved in the second step of leucine metabolism, catalyzing irreversible oxidative decarboxylation of α-ketoisocaproate.

Maximal activity of this enzyme assayed in mitochondrial extracts or in purified preparations requires the addition of CoA and NAD as cofactors (1,3) while with intact mitochondria and isolated liver cells no increase of the activity could be observed upon addition of these cofactors (4,5,6). Noda and Ichihara (4) proposed that α-ketoisocaproate oxidation to ketone bodies in rat liver mitochondria is controlled by the concentration of oxidazable substrate for the respiratory chain. In this work evidence is given for an inverse correlation between oxidative decarboxylation of α-ketoisocaproate in liver mitochondria and oxidative phosphorylation.

## MATERIALS AND METHODS

Mitochondria were prepared from livers of 300-350 g male Sprague-Dawley rats fed ad libitum. The livers were gently homogenized in a Tris-buffered 250 mM sucrose isolation medium (pH 7.2) to give a 20% homogenate. The homogenate was centrifuged at 800xg for 5 min and the supernatant solution was again centrifuged at 10,000xg for 10 min. The precipitate was washed in isolation medium and again centrifuged at 13,000xg for 10 min. The mitochondrial pellet was suspended in isolation medium. A typical reaction mixture for measuring oxidative decarboxylation of α-ketoisocaproate contained: 2 mM [1-$^{14}$C] sodium α-ketoisocaproate + 0.2 μCi (specific activity: 2 - 2.5 x $10^5$ dpm per

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μmol), 40 mM potassium glutamate, 3 mM ADP, 78 mM NaCl, 4.6 mM KCl, 1.2 mM $MgSO_4$, 15 mM $Na_2HPO_4$, and 2.5 - 4.5 mg mitochondrial protein. The final volume was 1 ml. Incubations were carried out at pH 7.4 in 25 ml siliconized glass flasks with a center well. A small test tube containing 0.2 ml 2-phenylethylamine was placed in the center well and the flask was tightly capped with a rubber stopper. The reaction was allowed to proceed for the time indicated at 37°C in a shaking water bath. The incubation was terminated by injecting 0.5 ml of 1 M citric acid through the rubber cap. Shaking was continued for 45 min to collect the $CO_2$ quantitatively. The tube then was transferred to a scintillation vial and counted in a LKB liquid scintillation counter. All samples were run in duplicate. Blank values were determined by carrying a buffer solution instead of mitochondria through an identical procedure. $^{14}CO_2$ release from [1-$^{14}$C] -α-ketoisocaproate during the incubation period is indicative for the extent of oxidative decarboxylation.

In some experiments mitochondria were preincubated for 10 min at 37°C in buffer before the incubation was started by the addition of [1-$^{14}$C] -α-ketoisocaproate. When indicated glutamate, ADP or ATP were present in the preincubation mixture. For ATP determination parallel incubations were performed. The reaction was stopped by adding 0.2 ml of 4 M perchloric acid and the mixture was immediately neutralized with 10 M KOH. ATP was measured enzymatically in the supernatant using hexokinase and glucose-6-phosphate dehydrogenase (7).

Mitochondrial respiration was measured in a Clark-type oxygen electrode at 37°C using 2.2 ml of the same incubation medium as described above. Mitochondrial protein was determined with the biuret method. [1-$^{14}$C] -α-ketoisocaproate was prepared from [1-$^{14}$C] L-leucine as described by Meister (8). [1-$^{14}$C] L-Leucine was purchased from Amersham Buchler KG, Braunschweig, FRG. Potassium atractylate was a product of Sigma Chemicals Co., St. Louis, Mo., USA. ADP, ATP, CoA and β-NAD were obtained from Boehringer Mannheim and glutamic acid was purchased from E. Merck, Darmstadt, FRG. All reagents were of analytical grade.

## RESULTS

In the absence of glutamate, ADP and ATP, α-ketoisocaproate was decarboxylated at a very low rate. Addition of glutamate, ADP or ATP separately did not enhance $CO_2$ formation and preincubation of the reaction mixture without α-ketoisocaproate was without effect. In the presence of both glutamate and ADP, without preincubation, a low rate of decarboxylation within the first 10 min was observed; between 10 and 20 min this rate increased twofold. When the mitochondria were preincubated in the presence of glutamate and ADP the rate of decarboxylation during the first 10 min of incubation was gretly enhanced (Fig. 1). The decarboxylation of α-ketoisocaproate was faster and nearly linear with time when mitochondria were preincubated (dashed line) while without preincubation $CO_2$ release was found to be biphasic, being slow in the first 10 min of incubation and then changing to a more rapid linear rate. Fig. 1B shows the time course of ATP synthesis during preincubation and subsequent incubation (dashed line) and during incubation without preceeding preincubation. In each case added ADP was almost completely converted to ATP but ATP synthesis proceeded at a slower rate when α-ketoisocaproate was present as oxidazable substrate.

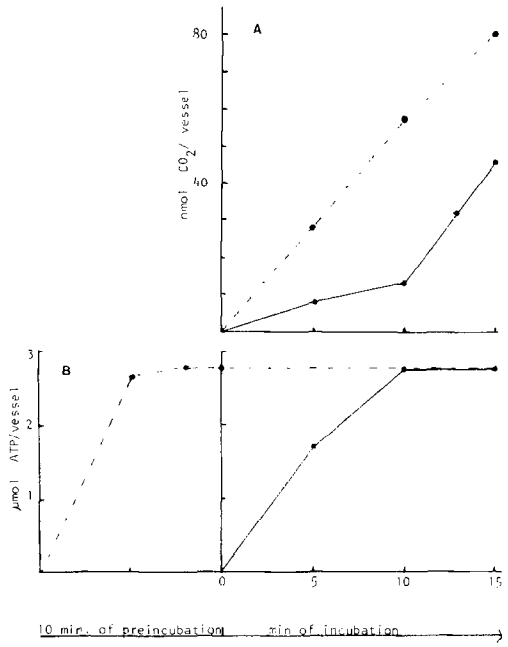


Figure 1 A. Effect of preincubation on oxidative decarboxylation of α-ketoisocaproate by rat liver mitochondria.
Experimental conditions were as described in MATERIALS AND METHODS. Preincubation was carried out with glutamate but without α-ketoisocaproate at 37°C for 10 minutes. The incubation was started by adding 2 mM α-ketoisocaproate. The figure shows the result of one typical experiment which has been reproduced five times. $CO_2$ formation during incubation with ( •---• ) and without ( •——• ) preincubation.

Figure 1 B. ATP synthesis of rat liver mitochondria.
ATP formation during preincubation and subsequent incubation ( •---• ) and during incubation without preceeding preincubation ( •——• ).

The decarboxylation rate in experiments without preincubation increased about threefold when ADP phosphorylation to ATP was completed.

The duration of the slow phase of decarboxylation depended on the amount of ADP added to the incubations (Fig. 2). Higher ADP concentrations prolonged the slow phase, which lasted as long as ATP formation continued. Following conversion of ADP to ATP the rates of decarboxylation of α-ketoisocaproate were found to be similar at all ADP concentrations tested. When no ADP was present a rapid but very short phase of decarboxylation was observed. These results suggest that oxidative decarboxylation of branched chain α-keto acids is inversely correlated with oxidative phosphorylation in liver mitochondria.

When ADP was present in excess and therefore not completely converted to ATP within the incubation period the rate of decarboxylation of $[1\text{-}^{14}C]$ -α-ketoisocaproate was low and nearly linear for 25 min (Con-

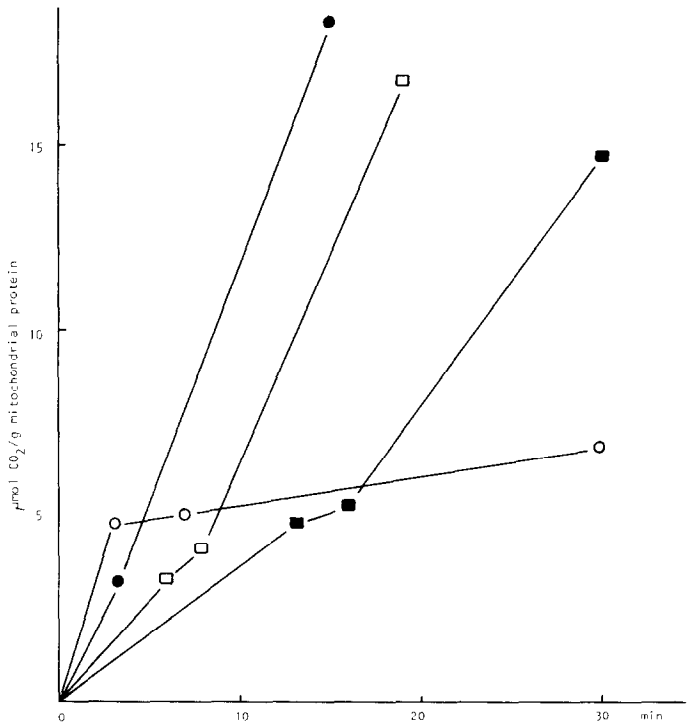


Figure 2. Effect of different ADP concentrations on decarboxylation of α-ketoisocaproate by rat liver mitochondria.
Experimental conditions were as described in MATERIALS AND METHODS. ADP concentration was varied from 0 to 4 mM. The incubation was started without a preceeding preincubation. The figure shows the result of one typical experiment which has been reproduced threetimes. Initial ADP concentrations were as follows:
No ADP ( o—o ), 1 mM ADP ( ●—● ), 2 mM ADP ( □—□ ), 4 mM ADP ( ■—■ ).

trol). Injection of atractylate which is known to inhibit the mitochondrial ADP/ATP carrier (9,10) immediately caused a marked stimulation of the decarboxylation rate. At the time of addition of atractylate the concentrations of ADP and ATP were 2.9 and 3.1 mM, respectively. Atractylate added at the beginning of the experiment did not enhance $CO_2$ release. This result also supports the concept that oxidative decarboxylation of branched chain α-keto acids in liver mitochondria is strongly dependent on the mitochondrial energy state.

## DISCUSSION

The results presented suggest an interaction between oxidative phosphorylation and oxidative decarboxylation of branched chain α-keto acids in rat liver mitochondria. As long as oxidative phosphorylation proceeds the rate of decarboxylation of $[1\text{-}^{14}C]$-α-ketoisocaproate is low. When ATP formation from added ADP is completed the rate of decarboxylation

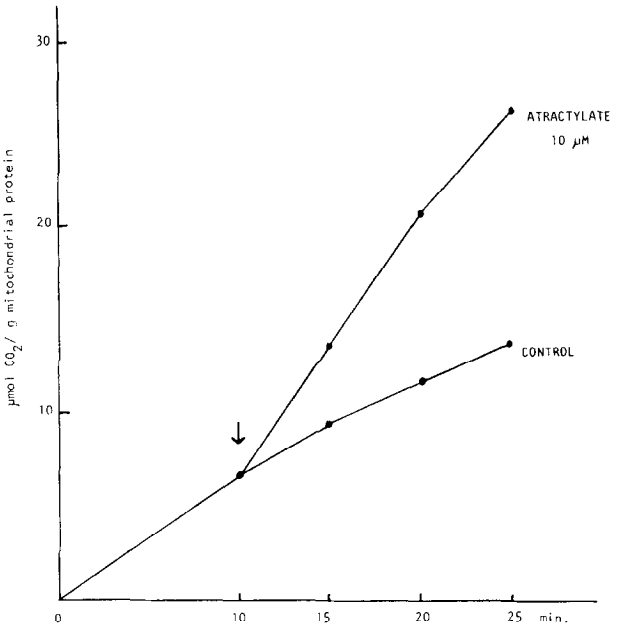


Figure 3. Effect of atractylate on decarboxylation of α-ketoisocaproate by rat liver mitochondria.
Experimental conditions were as described in MATERIALS AND METHODS. Initial ADP concentration was 6 mM. Atractylate at a final concentration of 10 mM was injected through the rubber cap after 10 min in parallel incubations (arrow). The figure shows the result of one typical experiment which has been reproduced three times.

increases markedly. A correlation between α-ketoisocaproate conversion to ketone bodies and the electron transport system has been reported also by Noda and Ichihara (4). Following ketone body formation from α-ketoisocaproate in respiring rat liver mitochondria they observed a biphasic increase with time, slow in the first 20 min and then rapid and linear with time. They concluded that both decrease of oxidizable substrate and increase of ATP concentration may be necessary for the rapid phase of ketone body formation. In our experiments the rate of α-ketoisocaproate oxidation has been recorded simultaneously with ATP formation as a parameter for oxidative phosphorylation. The concentration of oxidizable substrate was not limiting since glutamate was added in excess (40 mM). The rapid phase of decarboxylation began exactly when ATP formation terminated (Fig. 1) or when it was blocked by the addition of atractylate (Fig. 3). The concentration of ATP in the incubation mixture therefore appears not to be responsible for initiating the rapid phase of α-ketoisocaproate oxidation since addition of ATP had no effect on $CO_2$ production. Furthermore rapid $CO_2$ release was observed when no or only small amounts of ADP were present (Fig. 2). The disappearance of substrate cannot be the cause for the rapid phase of

decarboxylation as has been suggested by Noda and Ichihara (4), since in our experiments the decrease of the glutamate concentration was negligible. The rate of oxidative decarboxylation of branched chain α-keto acids in mitochondria appears to be inversely correlated with the rate of oxidative phosphorylation but not related to concentrations of substrates or cofactors such as NAD, CoA or ATP.

Phosphorylation of this enzyme complex has been reported to cause its inactivation (11,12,13). It is surprising therefore that high decarboxylation rates were observed at relatively high ATP concentrations (Fig. 1 and 2). From this result it can be assumed that the rapid phase of decarboxylation induced when oxidative phosphorylation is resting is not under control of this regulation mechanism. Parker and Randle (14) were also not able to inactivate the branched chain α-keto acid dehydrogenase complex by addition of 10 mM ATP, however, they observed a 50% loss of activity in the presence of 5 mM succinate.

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