EFFECT OF THENOYLTRIFLUOROACETONE ON OXYGEN CONSUMPTION AND ENERGY CONSERVATION IN ISOLATED RAT LIVER MITOCHONDRIA

Rune ULVIK
Laboratory of Clinical Biochemistry, University of Bergen, N-5016 Haukeland Sykehus, Norway

and

Inge ROMSLO
Department of Biochemistry, University of Bergen, N-5000 Bergen, Norway

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1. Introduction

Thenoyltrifluoroacetone (TTFA) is a well-known inhibitor of succinate oxidation [1–9]. Moreover, TTFA has been shown to uncouple oxidative phosphorylation and to inhibit ATP-supported reactions [10]. The effect of TTFA on succinate oxidation has been studied in submitochondrial particles [8,9] and in reconstituted succinate oxidase system [6,7], and it has been concluded that TTFA inhibits the succinate oxidase by chelating non-heme iron. The effect of TTFA on the energy-transducing system is far less understood, and the mechanism(s) by which it depresses oxidative phosphorylation and ATP-supported reactions, remains unknown.

So far, there are no quantitative data available to show differences in sensitivity towards TTFA of the succinate oxidase system and the energy-transducing system of intact mitochondria.

In the present paper it is shown that in intact mitochondria, TTFA at concentrations < 5 nmol/mg of protein markedly reduces succinate oxidation, with negligible effects on the energy-transducing system. On the other hand at concentrations > 10–15 nmol/mg of protein, TTFA functions as an uncoupler, as indicated by its ability to inhibit energy-linked accumulation of Ca^{2+}, to depress the P/O ratio, and to increase the passive proton conductance of the inner membrane.

2. Materials and methods

Mitochondria were prepared as previously described [11]. Mitochondrial respiration rates were determined by a Clark oxygen electrode [12]. Oxidative phosphorylation was determined as described by Grav et al. [13] except that bovine serum albumin was omitted and P_i was reduced to 1 mM.

Substrate-supported accumulation of Ca^{2+} was determined as previously described [14], and the Ca^{2+} loaded mitochondria were separated from the incubation medium by centrifugation through silicon oil [15].

External pH was determined by suspending mitochondria, approx. 10 mg of protein, at 25°C in a thermostated reaction chamber containing in a final volume of 3.0 ml: 0.25 M sucrose, 5 mM $MgCl_2$ and 1 mM Tris-buffer, pH 7.40. The H^+ concentration was measured by a semi-micro combination pH electrode (Radiometer, Copenhagen, Denmark) connected to a Radiometer pH meter, Model PHM 52.

3. Results

In the presence of succinate, State 4 respiration was progressively inhibited by increasing concentrations of TTFA, and the inhibition was apparent

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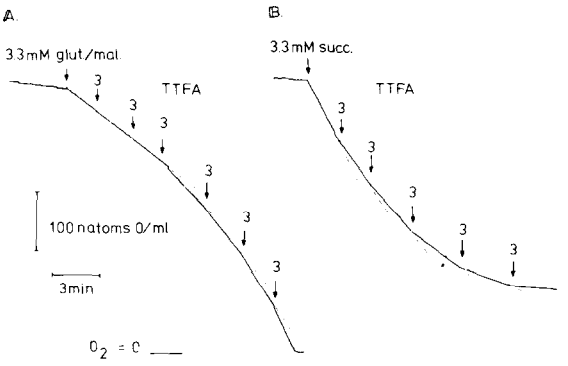

Fig.1. Effect of added TTFA on the respiration rate of rat liver mitochondria. The mitochondria were suspended in the standard incubation medium (see Methods) at 1.2 mg protein/ml in the absence (A) and presence (B) of 3.0 μM rotenone. Other additions were as indicated. TTFA was added in portions (arrows) of 3 nmol each.

already at concentrations $\approx$ 2–3 nmol/mg of protein. When mitochondria were incubated with glutamate plus malate, at concentrations below 5 nmol/mg of protein, TTFA had no effect on the State 4 respiration rate, whereas at concentrations > 10 nmol/mg of protein, there was a marked increase in State 4 respiration (fig.1).

Similar differences could be seen when measuring the State 3 respiration rate in the presence of increasing concentrations of TTFA (fig.2). Thus, whereas glutamate plus malate-supported State 3 respiration was only slightly reduced at concentrations of TTFA

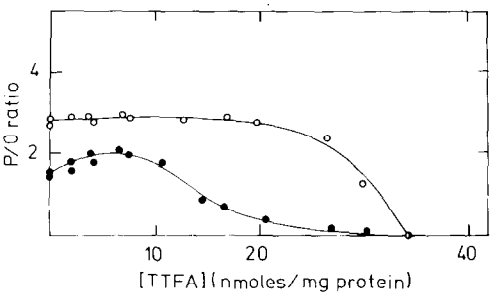

Fig.3. Effect of adding increasing concentrations of TTFA on the P/O ratio of rat liver mitochondria. Experimental conditions as described (see Methods). The substrates were 3.3 mM succinate (•) and 3.3 mM glutamate plus 3.3 mM malate plus (○).

< 10 nmol/mg of protein, succinate-supported State 3 respiration leveled off at concentrations of TTFA < 5 nmol/mg of protein. This decrease in succinate-supported State 3 respiration was not accompanied by a proportional decrease in the rate of phosphorylation, and as can be seen (fig.3), this resulted in an apparent slight increase in the P/O ratio.

At concentrations of TTFA > 15–20 nmol/mg of protein, the mitochondria were uncoupled, irrespective of substrates (fig.3).

Essentially similar results were obtained when utilizing the substrate-supported, energy-linked accumulation of calcium as a parameter to test the energy-coupling of the mitochondria, i.e. in the presence of succinate, calcium accumulation began

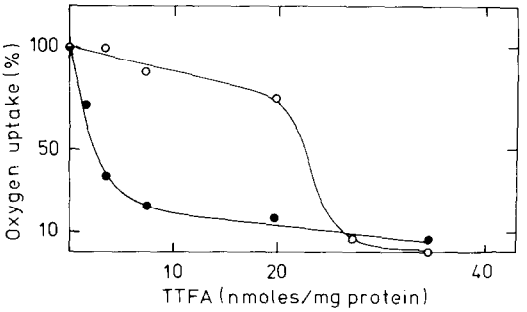

Fig.2. Effect of adding increasing concentrations of TTFA on State 3 respiration in rat liver mitochondria with succinate (•) and glutamate plus malate (○) as substrates. State 3 respiration was initiated with 0.83 mM ADP. 100% oxygen uptake was 110 and 84 natoms O/min/mg protein with 3.3 mM succinate and 3.3 mM glutamate plus 3.3 mM malate respectively.

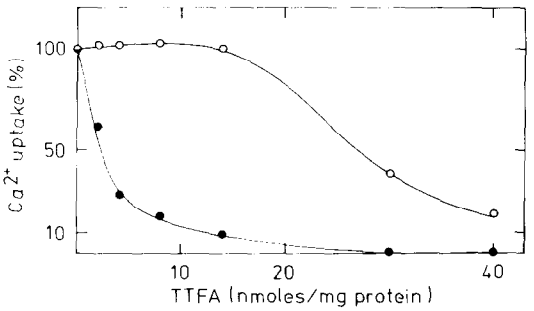

Fig.4. Effect of adding increasing concentrations of TTFA on succinate (•) and glutamate plus malate (○) supported accumulation of Ca^{2+}. Experimental conditions as described (see Methods). TTFA was added simultaneously with 0.2 mM Ca^{2+}. 100% uptake was 98 and 87 nmol/mg protein for succinate and glutamate plus malate supported accumulation respectively.

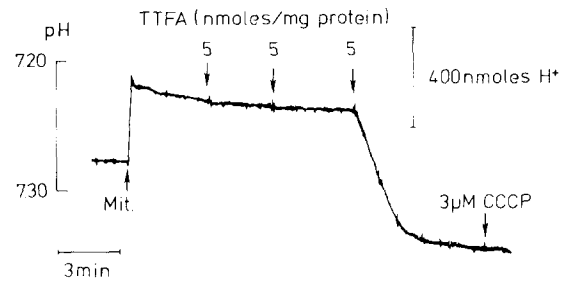

Fig.5. Effect of adding increasing concentrations of TTFA on the external pH in suspended rat liver mitochondria. Experimental conditions as described (see Methods).

to level off at 2–3 nmol TTFA/mg of protein, whereas in the presence of glutamate plus malate, approx. 20 nmol TTFA/mg of protein were necessary to depress the accumulation of calcium (fig.4).

From the results presented in fig.5, it is seen that TTFA at concentrations > 10–15 nmol/mg of protein equilibrates the transmembrane pH gradient by increasing the proton conductance of the inner membrane essentially as reported for other uncouplers [16].

4. Discussion

TTFA at concentrations > 25–50 nmol/mg of protein has been shown to depress succinate oxidation and oxidative phosphorylation in respiring mitochondria [10]. From the date reported in the present study, it has been possible to discriminate between the effect of TTFA on the succinate oxidation and the energy-transducing system, and it is shown that TTFA effectively inhibits succinate oxidation at concentrations well below those so far reported. Furthermore, the succinate-oxidase sytem is shown to be 2–3 times more sensitive to TTFA than is the energy-transducing system (figs.3 and 4), and as can be seen (fig.5) the uncoupling is due to an increase in the passive proton conductance of the inner membrane, essentially as reported for other uncouplers [15].

As already mentioned, the mechanism by which TTFA inhibits succinate oxidation has been attributed to its ability to chelate non-heme iron of the succinate-dehydrogenase complex [1–3, 5–10], presumably by a strict steric specificity on the succinate-dehydrogenase cytochrome *b* system [9]. However, the inhibition by TTFA is readily reversible. On centrifugation and washing once with fresh buffer, the succinate oxidase activity of a TTFA-inhibited preparation can be restored to that of a control preparation [4]. Moreover, the inhibitory action of $Fe(TTFA)_3$ equals that of TTFA, and it has been shown that $Fe(TTFA)_3$ is unstable in P_i at pH 7.4 [4]. Also, the characteristic $Fe(TTFA)_3$-absorption band in the region of 335 nm has not been observed in TTFA-inhibited mitochondria [4]. Thus, the suggestion that TTFA functions by chelating non-heme iron might be held in some doubt until more direct evidence is available.

The effect of TTFA on the energy-transducing system may be a non-specific effect due to its aromatic and lipophilic characteristics [10].

As reported by Nelson et al. [8,9], in submitochondrial particles TTFA at concentrations > 60 nmol/mg of protein activates an 'electron sink' in the succinate dehydrogenase cytochrome *b* segment of the electron transport system. Whether or not the activation by TTFA of a similar 'electron sink' in intact mitochondria may explain the anomalous P/O ratios obtained with succinate, remains to be determined.

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