Short communication

Thenoyltrifluoracetone Induces Lipid Peroxidation in Rat Liver Mitochondria

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Abstract. Addition of thenoyltrifluoracetone to mitochondria oxidizing NAD-dependent substrates induced lipid peroxidation and uncoupling of oxidative phosphorylation. Butylated hydroxytoluene, an antioxidant, prevented accumulation of lipid peroxidation products but failed to prevent deenergization of the mitochondria.

Key words: Mitochondria — Lipid peroxidation — Thenoyltrifluoracetone

Introduction

Thenoyltrifluoracetone (TTFA) is widely used as an effective inhibitor of mitochondrial succinate dehydrogenase. At the same time it has been shown that at a concentration range from 50 to 200 μmol/l TTFA induced uncoupling of oxidative phosphorylation in mitochondria oxidizing NAD-dependent substrates (Warshaw et al. 1966). In studying the mechanism underlying the processes of succinate-induced inhibition of lipid peroxidation, we used TTFA as part of some protocols. The observation presented herein shows that besides uncoupling, TTFA activates lipid peroxidation in mitochondria oxidizing NAD-dependent substrates.

Materials and Methods

All chemicals were obtained from commercial sources and were reagent grade. Prior to use, sucrose for mitochondrial media was deionized on Dowex (Serva). TTFA, Tris, thiobarbituric acid (TBA), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and butylated hydroxytoluene (BHT) were obtained from Sigma, ethylene glycol bis (2-aminoethylether)-N,N'-tetraacetic acid (EGTA) was from Serva.

Rat liver mitochondria were prepared by the conventional procedure in 0.25 mol/l sucrose, containing 5 mmol/l Tris-HCl (pH 7.5) and 0.2 mmol/l EGTA. EGTA was omitted from the final washing solution, and sedimented mitochondria were suspended in the same solution at 70–80 mg protein/ml. The standard incubation medium contained 0.1

mol/l KCl, 0.1 mol/l sucrose, 2 mmol/l KH_2PO_4, 5 mmol/l pyruvate, 5 mmol/l malate, 5 mmol/l Tris-HCl, pH 7.5. Oxygen consumption was monitored with a Clark-type electrode. Lipid peroxidation was estimated using the TBA test (Ohkava et al. 1979). Protein was determined by the method of Lowry et al. (1951). All incubations were carried out at 30 °C.

Results and Discussion

When TTFA was added to mitochondria oxidizing pyruvate with malate, a time dependent accumulation of TBA active products was observed (Fig. 1, filled circles). It is well established that succinate added to mitochondrial suspension significantly inhibits lipid peroxidation promoted by different prooxidants. This inhibitory effect was thought to result from reduction of ubiquinone in mitochondrial respiratory chain (Cavallini et al. 1984) since ubiquinone, mainly in reduced state, may act as an antioxidant protecting a number of cellular membranes from free radical damage (Beyer 1990). To check whether TTFA-induced lipid peroxidation is or not caused by abolishment of protective action of endogenous succinate, malonate, another inhibitor of succinate dehydrogenase, was used along with TTFA (Fig. 1, open circles). As can be seen, inhibition of endogenous succinate oxidation by malonate did not induce accumulation of lipid peroxidation products.

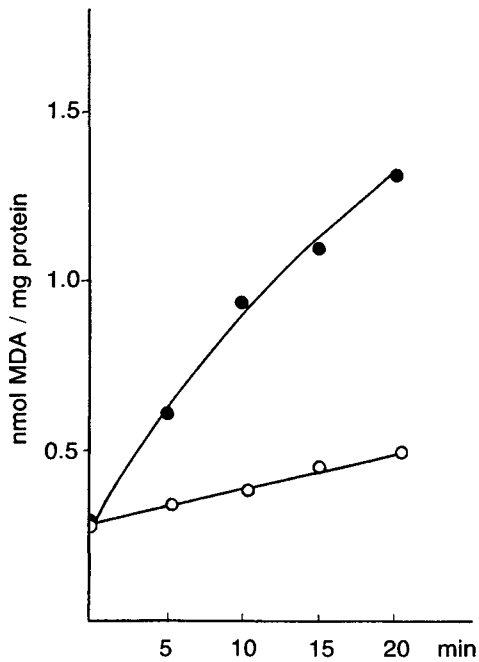

Figure 1. Effects of TTFA (•) and malonate (○) on TBA-active products accumulation. TTFA, 60 μmol/l; malonate, 5 mmol/l; mitochondria, 2 mg/ml.

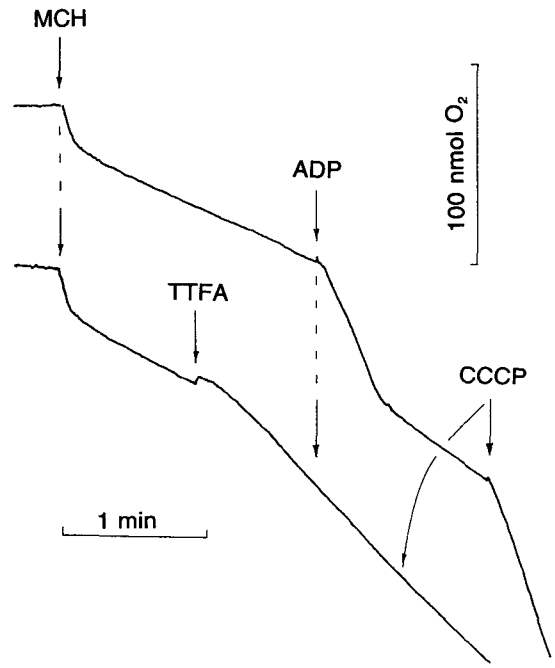

Figure 2. Effect of TTFA on respiration of mitochondria. ADP, 150 μmol/l; CCCP, 1 μmol/l; TTFA, 60 μmol/l; mitochondria (MCH), 2 mg/ml.

Fig. 2 shows the effect of TTFA on the respiration of mitochondria oxidizing pyruvate with malate. Oxidation of endogenous succinate was prevented by addition of malonate (3 mmol/l). Under the conditions employed, TTFA was shown to stimulate State 4 respiration. ADP had no effect on the respiration rate, suggesting uncoupling of oxidative phosphorylation. This finding appears to be in a close agreement with the uncoupling properties of TTFA (Warshaw et al. 1966). As seen from Fig. 2, uncoupler CCCP failed to stimulate oxygen consumption after TTFA addition and at the same time, the rate of respiration was lower than in the absence of TTFA; this points to a partial inhibition of respiratory chain enzymes. The traces shown were obtained with pyruvate plus malate, but are typical representatives of many experiments with other NAD-dependent substrates such as glutamate plus malate of β-hydroxybutyrate.

It has been shown that lipid peroxidation products can uncouple oxidative phosphorylation and inhibit respiration (Ceconi et al. 1988). Fig. 3 illustrates the dependences of accumulation of TBA-active products and State 4 respiration rate on TTFA concentration. Increasing concentrations of TTFA (from 6 to 200 μmol/l) progressively activate lipid peroxidation (filled circles), following a saturation type curve. As can be seen, the accumulation of TBA-active products coincides with the increase of oxygen consumption (open circles); this can be viewed as an evi-

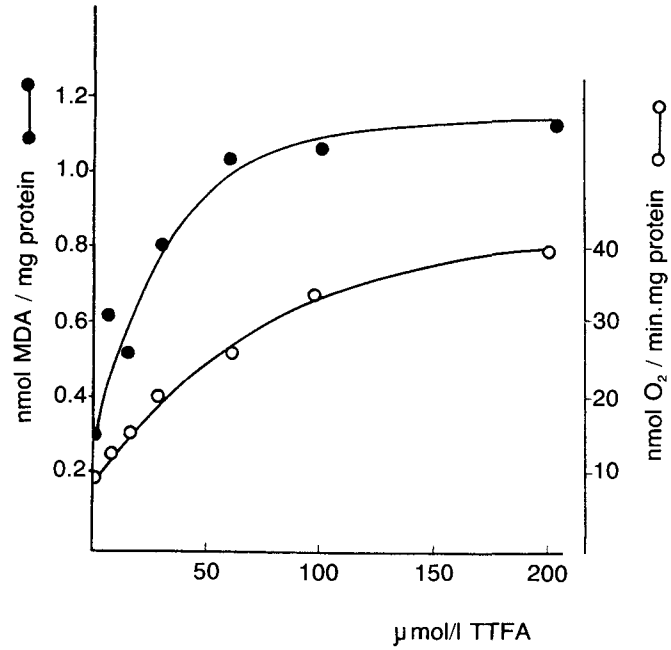

Figure 3. Effects of increasing concentrations of TTFA on TBA-active products accumulation (•) and the rate of respiration (○). Lipid peroxidation was estimated after 20 min of incubation. Mitochondria, 2 mg/ml.

Table 1. The effects of BHT on TTFA-induced lipid peroxidation and the rate of mitochondrial respiration.

Additions	TBA-active products* (nmol/l MDA/mg protein)	Rate of respiration** (nmol/l O_2/min mg protein)
BHT 20 mmol/l	0.08 ± 0.03	12.3 ± 2.4
TTFA 50 mmol/l	0.76 ± 0.09	28.7 ± 3.6
BHT + TTFA	0.08 ± 0.03	29.4 ± 2.2

Results are mean ± S.E.M.
*Aliquots of 0.2 ml for MDA determination were taken 10 min after TTFA addition.
**The rate of respiration was estimated 1 min after TTFA addition

dence for the causative role of lipid peroxidation in the uncoupling of oxidative phosphorylation.

To check whether uncoupling after TTFA addition is due to accumulation of lipid peroxidation products, the influence of antioxidant BHT on TTFA-induced

activation of respiration was analyzed. Data summarized in Table indicate that the addition of BHT prior to TTFA completely blocks the accumulation of TBA-active products, having no effect on oxygen consumption. This indicates that the uncoupling of oxidative phosphorylation is not mediated by peroxidative processes.

On the other hand, Chance et al. (1979) have reported that mitochondria are one of the main intracellular sources of superoxide $O_2^{\cdot-}$ and H_2O_2 under physiological conditions, and that uncoupled mitochondria generate $O_2^{\cdot-}$ at much higher rates than do well-coupled mitochondria. However, under conditions employed in the present experiments uncoupling of mitochondria by CCCP did not bring about a rise of lipid peroxidation product concentrations (data not shown); thus uncoupling of mitochondria by TTFA can be ruled out as being the cause triggering lipid peroxidation.

Recently it has been shown that in the presence of TTFA succinate initiates lipid peroxidation without the need for iron or iron helate (Glinn et al. 1991). However, as mentioned above another inhibitor of succinate dehydrogenase, malonate, failed to induce accumulation of lipid peroxidation products, indicating that TTFA initiates peroxidation directly. Apparently, this effect is attributed to iron helating properties of TTFA (Warshaw et al. 1966) that facilitates autoxidation of Fe^{2+} and increases peroxidation rate (Harris and Aisen 1973).

The results presented herein show one of the side effects of TTFA which obviously should be taken into account. Apparently, the prooxidant effect of TTFA is weak in comparison with those of potent prooxidants (Fe^{2+}-ascorbate, organic hydroperoxides), but nevertheless it may be a source of artifacts, especially when investigating the antioxidant properties of succinate of estimating Ca^{2+} transport supported by NAD- dependent substrate oxidation.

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Final version accepted March 9, 1993