Inhibition of Palmityl Carnitine Oxidation in Rat Liver Mitochondria by

*Tert*-Butyl Hydroperoxide

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Summary

Mitochondria as an energy generating cell device are very sensitive to oxidative damage. Our previous findings obtained on hepatocytes demonstrated that Complex I of the respiratory chain is more sensitive to oxidative damage than the other respiratory chain complexes (Drahota et al. 2005). We present additional data on isolated mitochondria showing that at a low (200 µM) tert-butyl hydroperoxide (tBHP) concentration palmityl carnitine oxidation is strongly depressed, while oxidation of the flavoprotein-dependent substrate-succinate is not affected and neither is ATP synthesis inhibited by tBHP. In the presence of tBHP, the respiratory control index for palmityl carnitine oxidation is strongly depressed, but when succinate is oxidized the respiratory control index remains unaffected. These findings thus show that for the regeneration process in the necrotic liver damaged by oxidative stress, flavoprotein-dependent substrates could be an important nutritional factor.
Liver cells represent an important factor in the traffic of lipids as a nutritional energy source for animal organisms (Havel 1987). Some lipids, namely short and medium chain fatty acids entering the liver through the portal vein, are oxidized directly in hepatocytes by mitochondria to cover their energy demands.

It is now commonly accepted that mitochondria are not only an important generator of reactive oxygen species (Boveris and Chance 1973, Chance et al. 1979), but also an important target of their action (Turrens and Boveris 1980, Kowaltowski and Vercesi 1999, Nulton-Presson and Szweda 2001, Turrens 2003). Liver tissue is especially sensitive to various toxic agents that induce processes in which reactive oxygen species play an important role (Shu et al. 1997, Knight et al. 2003).

There are many studies describing peroxidative damage of liver mitochondria (Kennedy et al. 1992, Kowaltowski and Vercesi 1999, Nulton-Persson and Szweda 2001, Lin et al. 2002) and hepatocytes (Masaki et al. 1989, Nieminen et al. 1997, Kmoničková et al. 2001). However, there are not sufficient comparative data to evaluate the sensitivity of various mitochondrial dehydrogenases to peroxidative damage.

This is why in our previous experiments on isolated hepatocytes we studied the peroxidative damage of mitochondrial enzymes induced by tert-butyl hydroperoxide (tBHP). In these studies we used tBHP as the prooxidant because, unlike hydrogen peroxide, it is not degraded by catalase (Chance et al. 1979) and consequently its peroxidative effect can be studied over a longer period of incubation. We found that oxidation of NADH-dependent substrates is extremely sensitive to peroxidative damage and also that oxidation of flavoprotein dependent substrates is partially reduced (Drahota et al. 2005, Křiváková et al. 2007).

It has not been fully elucidated to what extent the toxic effects of prooxidants are due to the direct action on mitochondrial enzymes and to what extent secondary radical metabolites
formed in cytosol (Kennedy et al. 1992) could also be involved in peroxidative damage when isolated hepatocytes are used. Following up on our previous experiments on hepatocytes, therefore, in this study we measured isolated mitochondria and we tested to what extent fatty acid oxidation and succinate oxidation are affected by tBHP. In order to obtain additional data, we tested the effect of tBHP on palmityl carnitine and succinate oxidation on isolated liver mitochondria under the same experimental conditions. We compared the effect of tBHP on palmityl carnitine and succinate oxidation, because oxidation of palmityl carnitine involves two mitochondrial dehydrogenases, a NADH- and a flavoprotein-dependent one, whereas succinate is oxidized only by the flavoprotein-dependent dehydrogenase.

Liver mitochondria were isolated from male albino Wistar rats (b.w. of 220-230 g) by differential centrifugation as described previously (Ješina et al. 2004). Oxygen uptake was measured with the High Resolution Oxygraph2K (OROBOROS, Austria). Measurements were taken at 30°C in 2 ml of incubation medium containing 100 mM KCl, 10 mM Tris HCl, 4 mM K-phosphate, 3 mM MgCl₂, 1 mM EDTA, 0.5 mg/ml fatty acid free bovine serum albumin, pH 7.4. The rate of oxygen uptake was expressed as pmol/s/mg protein. Oxygen uptake curves are presented as the first negative derivation of the oxygen tension changes.

Fig. 1 demonstrates the experimental conditions used for evaluation of the tBHP effect on the oxidation of palmityl carnitine and succinate performed by rat liver mitochondria. In contrast to other respiratory substrates which are used in high (millimolar) concentrations, palmityl carnitine must be tested at micromolar concentrations, because at higher concentrations it exerts a detergent-like effect on mitochondrial membranes. We used 5 μM palmityl carnitine and 2.5 mM malate as a sparker. After the addition of the ADP, the oxygen uptake was highly activated (five-fold, Fig. 1A, Table 1), indicating that mitochondria are well coupled and that the concentration used of the palmityl carnitine had no detergent-like effect. When palmityl carnitine was oxidized, the respiratory rate decreased and another
portion of palmityl carnitine induced a similar response in oxygen uptake (Fig. 1A). The addition of rotenone, an inhibitor of Complex I, completely inhibited palmityl carnitine oxidation (not shown). The addition of succinate after palmityl carnitine again increased the rate of oxygen uptake to values about 30-50% higher than those obtained with palmityl carnitine and malate (Fig. 1A and Table 1). When tBHP was added after palmityl carnitine was oxidized, then subsequent addition of palmityl carnitine induced a much smaller increase in the respiratory rate (Fig. 1B). However, subsequent addition of succinate demonstrated that the rate of succinate oxidation was not depressed by tBHP (Fig. 1B, and Table 1). The same results were obtained when the effect of tBHP on the oxidation of succinate was tested in the absence of palmityl carnitine and malate (Fig 1C, D, and Table 1).

From our data it is evident that fatty acid oxidation is more sensitive than oxidation of succinate to peroxidative damage (Fig. 1, and Table 1). We may also conclude based on our experiment that under conditions when fatty acid oxidation is highly depressed by tBHP (Fig. 1C, D), the ATP synthase is active, because the respiratory rate induced by ADP is not affected (Table1). Our data thus confirm our previous results obtained on isolated hepatocytes (Drahota et al. 2005, Křiváková et al. 2007), demonstrating that Complex I is the most sensitive part of the mitochondrial respiratory chain to peroxidative damage. Experiments on isolated mitochondria further indicate that the inhibition of Complex I is not necessarily connected with the collapse of membrane potential if flavoprotein dependent substrates are accessible. These conclusions are also supported by our previous data on isolated hepatocytes (Lábajová et al. 2006), demonstrating that the dissipation of the mitochondrial membrane potential by tBHP, when pyruvate and malate are used as respiratory substrates, can be fully recovered by succinate.

Our data showing that fatty acid oxidation is highly sensitive to oxidative damage could be important information for the treatment of necrotic liver tissue because most hepatotoxic
agents are known to be reactive oxygen species inducers (Knight et al. 2003, Sundari et al. 1997). For the activation of the regeneration process, therefore, flavoprotein-dependent substrates could play an important role.

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References


**Text to Fig. 1**

**Fig. 1.** Respiration of rat liver mitochondria in the presence of palmityl carnitine and succinate: Mitochondria (0.2 mg protein/ml) were incubated in a KCl medium with 0.5 mg of bovine serum albumine. Where indicated, 2.5 mM malate (MAL), 5 µM palmityl carnitine (PC), 1.5 mM ADP, 10 mM succinate (SUC) and 200 µM t-butyl hydroperoxide (tBHP) were added. The respiratory control index (RCI) in part A was 5.2 and 5.0; in B 4.8 and 1.8; in C 4.3; and in part D, 3.9.
Table 1. Inhibition of palmityl carnitine and succinate oxidation by tBHP.

<table>
<thead>
<tr>
<th>Additions</th>
<th>pmole oxygen/s/mg protein</th>
<th>+tBHP/-tBHP</th>
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<tr>
<td>Palmityl carnitine + malate</td>
<td>117.0 ± 14.0</td>
<td></td>
</tr>
<tr>
<td>Palmityl carnitine + malate + ADP</td>
<td>601.5 ± 27.6</td>
<td></td>
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<tr>
<td>Palmityl carnitine + malate + ADP + tBHP</td>
<td>245.7 ± 27.4</td>
<td>0.40 (p≤0.001)</td>
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Succinate + ADP                                                907.6 ± 53.0  
Succinate + ADP + tBHP                                   883.9 ± 29.9  0.87 (n.s.)

Mitochondria were incubated as described in the legend to Fig. 1. The palmityl carnitine used was 5 µM, malate 2.5 mM, ADP 1.5 mM, succinate 10 mM and tert-butyl hydroperoxide 200 µM. The data presented depict the average± SEM from six liver mitochondrial preparations isolated from six adult male rats. The statistical significance was analysed using t-test.
Fig. 1