# Inhibition of pyruvate oxidation in rat liver mitochondria by dihydrolipoic acid

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We have studied the effect of dihydrolipoic acid (DHLA) on the respiration of rat liver mitochondria oxidizing pyruvate, since DHLA is a potent inducer of the mitochondrial permeability transition (MPT) when pyruvate is used as a respiratory substrate. DHLA did not affect mitochondrial respiration in state 2, but to a similar extent (by approximately 30%) inhibited ADP-stimulated (state 3) as well as uncoupler-stimulated respiration. The obtained results indicated that DHLA did not change the inner membrane permeability to protons, but reduced the activity of the components of the respiratory system. The inhibition was not due to MPT, since cyclosporin A did not change the degree of inhibition.

**Key words:** dihydrolipoic acid, mitochondrial respiration, permeability transition

## INTRODUCTION

α-Lipoic (LA) is a naturally occurring compound known as a cofactor for several enzymes. It is essential as an acyl carrier in the oxidative decarboxylation of the α-keto acids (pyruvate, 2-oxoglutarate and branched-chain 2-oxodehydrogenase complexes) and as an aminomethyl carrier in the glycine-cleavage enzyme systems [1]. In cells *in vitro*, LA is rapidly taken up, reduced to DHLA and then released [1]. Evidence is accumulating that free LA/DHLA can act at various levels in biochemical pathways. Recently, LA/DHLA has gained considerable interest in the treatment of diabetes [2], neurodegenerative disorders and as a radioprotective agent [3, 4]. LA/DHLA protects against ischemia/reperfusion injury as well [5]. These beneficial effects are claimed to be due to antioxidant properties of LA/DHLA. However, it is known that DHLA exhibits prooxidant properties also [3]. Both reduced and oxidized forms of LA were found to stimulate $Ca^{2+}$ release [6] and to induce the permeability transition (MPT) in rat liver mitochondria (RLM) [7]. It was reported that LA/DHLA inhibited several enzymes [1]. In this work, we investigated the effect of DHLA on mitochondrial respiration.

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## MATERIALS AND METHODS

RLM were isolated by a standard procedure [8] using a medium containing 220 mM mannitol, 70 mM sucrose, 10 mM Hepes/Tris (pH 7.4), 1 mM EGTA and 0.5 mg/ml BSA. Mitochondria were washed 2–3 times using the same medium without EGTA and albumin. Mitochondrial respiration was measured with a Clark-type oxygen electrode combined to an Orabora data acquisition and analysis system. The experiments were performed at 25 °C in a medium containing 100 mM KCl, 2 mM $KH_2PO_4$, 10 mM Hepes (pH 7.4), 5 mM of pyruvate, 10 μM $CaCl_2$, and 1 mg/ml mitochondria. The rate of mitochondrial respiration in state 3 was registered after addition of 2 mM ADP. Uncoupled respiration rate was obtained by addition of 100 nM carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) instead of ADP.

## RESULTS

We have previously shown that DHLA effectively induced MPT under the experimental conditions used in this study, however, MPT occurred only following approximately 3 minutes of preincubation of mitochondria with 10 μM $Ca^{2+}$ and 20 μM DHLA. In this work, we studied the effect of DHLA on oxygen consumption in RLM respiring with pyruvate immediately after DHLA addition. The results

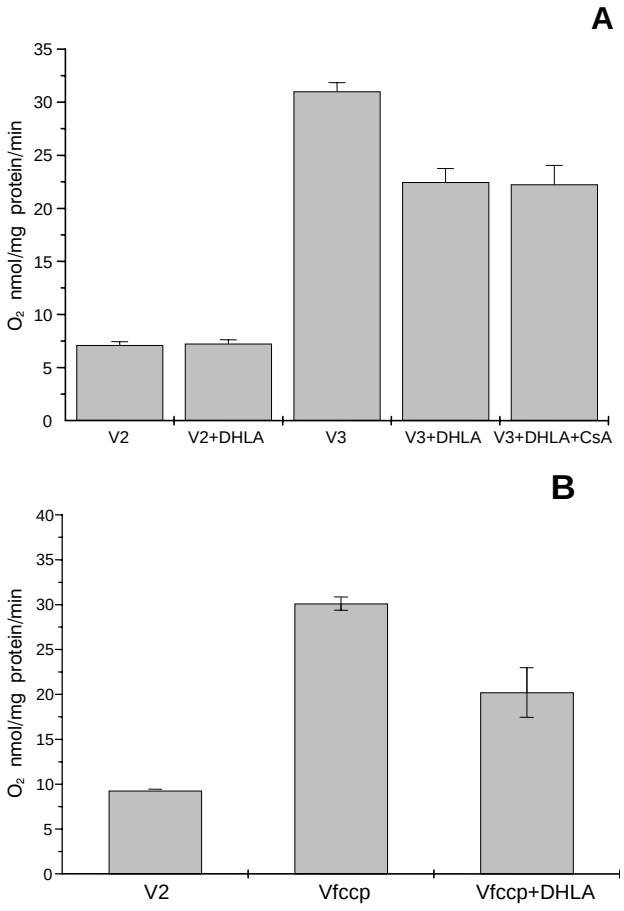


Figure. The effect of DHLA on respiration of liver mitochondria
A – respiration in state 2 and state 3. V2 indicates respiration rate in state 2 (without addition of ADP), V3 – respiration rate in state 3 (in the presence of 2 mM ADP); +DHLA indicates the presence of 20 μM DHLA, and +CsA indicates the presence of 0.5 μM CsA added to incubation medium; B – uncoupled respiration: 100 nM FCCP was added instead of ADP

showed (Fig. 1, A) that there was no effect of DHLA on respiration rate in state 2 (in the absence of ADP) as long as MPT was not induced.

However, DHLA caused a decrease in respiration rate in the presence of ADP (Fig. 1A). The inhibition of respiration was not due to MPT, since the specific inhibitor of MTP cyclosporin A (CsA), had no effect on the extent of inhibition of mitochondrial respiration by DHLA (Fig. 1A). In these experiments, CsA itself did not affect the rate of mitochondrial respiration (data not shown). It is evident from results shown in Fig. 1 that DHLA inhibited the uncoupled respiration (Fig. 1B) to the same extent (by 33 ± 8%) as state 3 respiration (by 29 ± 4%; Fig. 1A). The obtained data clearly indicate that the respiratory subsystem in liver mitochondria oxidizing pyruvate is inhibited by DHLA. The inhibition of the components of the phosphorylation machinery (ATP/ADP carrier, ATP synthase and phosphate carrier) by DHLA is not excluded, however, this study does not provide sufficient evidence for making conclusions on this point.

## DISCUSSION

The effect of DHLA on mitochondrial respiration was studied with pyruvate as a respiratory substrate, because we have earlier observed that DHLA is a potent inducer of MPT when pyruvate is used as a respiratory substrate [9]. We report in this paper that DHLA inhibits the activity of the oxidative phosphorylation system in liver mitochondria. DHLA did not change mitochondrial respiration with pyruvate in state 2. That finding implies that DHLA does not change inner membrane permeability to protons in mitochondria. However, it significantly inhibits the respiration both in state 3 and in the uncoupled state of mitochondria. The latter finding indicates that DHLA reduces the activity of enzymes involved in the oxidation pathway of pyruvate – pyruvate carrier, pyruvate dehydrogenase, and/or the complexes of the respiratory chain. The targets of DHLA within this pathway, as well as possible interaction of DHLA with the components of the phosphorylation subsystem, remain to be determined in further investigation. It was obvious from the obtained results that the observed DHLA effect on respiration was not due to MPT, since CsA did not prevent the inhibition.

The high sensitivity of mitochondrial respiration with pyruvate to DHLA could be related to the presence of dihydrolipoamide dehydrogenase in the pyruvate dehydrogenase (PDH) complex. There is some probability that excessive amounts of DHLA may reduce the activity of dihydrolipoamide dehydrogenase by the mechanism of product inhibition. On the other hand, an indirect effect of DHLA on PDH is also possible, since DHLA is able to increase superoxide production in rat liver mitochondria (Š. Morkūnaitė et al., submitted data) and PDH is known to be very sensitive to reactive oxygen species [10]. Another possible reason for the decrease in the activity of the pyruvate oxidizing system is depletion of the mitochondrial pool of reduced pyridine nucleotides induced by DHLA. We have previously shown that the amount of reduced pyridine nucleotides was almost completely ecxausted within a few minutes after addition of DHLA to mitochondria oxidizing pyruvate [11]. Complex I is one of the key enzymes responsible for pyruvate oxidation in mitochondria. Therefore a severe reduction in the amount of NADH, the substrate of Complex I, may lead to an appreciable inhibition of the mitochondrial respiration rate.

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## DIHIDROLIPOINĖS RŪGŠTIES POVEIKIS ŽIURKĖS KEPENŲ MITOCHONDRIJŲ KVĖPAVIMUI

S a n t r a u k a

Šiame darbe nustatėme dihidrolipoinės rūgšties (DHLA) poveikį žiurkės kepenų mitochondrijų kvėpavimo greičiui, kai kvėpavimo substratu naudojamas piruvatas. Šį substratą oksiduojančiose mitochondrijose DHLA lengvai indukuoja nespecifinio pralaidumo porą (MPT). Nustatyta, kad 20 μM DHLA neveikia mitochondrijų kvėpavimo antroje metabolinėje būsenoje, tačiau vienodai inhibuoja tiek ADP, tiek skyrikliu FCCP stimuliuotą kvėpavimą. Tai rodo, kad DHLA nekeičia mitochondrijų vidinės membranos laidumo protonams, bet inhibuoja kvėpavimo posistemės komponentų aktyvumą. Kvėpavimo inhibicija nebuvo susijusi su MPT, nes ciklosporino A priedas nesumažino inhibicijos laipsnio.