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Effect of α-lipoic acid and dihydrolipoic acid on ischemia/reperfusion injury of the heart and heart mitochondria

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Abstract

The aim of the present study was to evaluate a possible interference of α-lipoic acid (LA) or its reduced form (dithiol dihydrolipoic acid = DHLA) in the cardiac ischemia/reperfusion injury both at the level of the intact organ and at the subcellular level of mitochondria. In order to follow the effect of LA on the ischemia/reperfusion injury of the heart the isolated perfused organ was subjected to total global ischemia and reperfusion in the presence and absence of different concentrations of LA. Treatment with 0.5 μM LA improved the recovery of hemodynamic parameters; electrophysiological parameters were not influenced. However, application of 10 μM LA to rat hearts further impaired the recovery of hemodynamic functions and prolonged the duration of severe rhythm disturbances in comparison to reperfusion of control hearts. Treatment of isolated mitochondria with any concentration of DHLA could not prevent the impairment of respiratory-linked energy conservation caused by the exposure of mitochondria to 'reperfusion' conditions. However, DHLA was effective in decreasing the formation and the existence of mitochondrial superoxide radicals ($O_2^{\cdot-}$). Apart from its direct $O_2^{\cdot-}$-scavenging activities DHLA was also found to control mitochondrial $O_2^{\cdot-}$ formation indirectly by regulating redox-cycling ubiquinone. It is suggested that impairment of this mitochondrial $O_2^{\cdot-}$ generator mitigates postischemic oxidative stress which in turn reduces damage to hemodynamic heart function.

Keywords: Heart; Mitochondria; Dihydrolipoic acid; α-Lipoic acid; Superoxide radical; Ubisemiquinone

1. Introduction

Ischemia/reperfusion of the heart is assumed to cause an unphysiological production of oxygen-free radicals resulting in myocardial stunning, ventricular arrhythmias and a great variety of biochemical alterations [1–6]. Sulfhydryl groups are highly sensitive to oxygen radicals and other prooxidants. The maintenance of the physiological thiol redox status is a prerequisite to ensure the function of a great variety of enzymes and carrier proteins. Efforts to control a fall of thiol tissue levels were therefore assumed to be effective in the reduction or even prevention of organ damage resulting from ischemia/reperfusion. Lipoic acid (LA) a co-factor of mitochondrial dehydrogenase complexes was increasingly reported to have antioxidant properties. Its reduced form, the dithiol dihydrolipoic acid (DHLA), which is in equilibrium with tissue LA was shown to be an effective scavenger of water- and lipid-soluble radicals [7]. Furthermore, this compound is also discussed to exert stabilizing effects on the cellular thiol redox status [8,9]. The aim of the present study was to clarify the following questions:

1. Can α-lipoic acid/dihydrolipoic acid reduce myocardial ischemia/reperfusion injury when acutely administered before ischemia to isolated hearts?

2. Is the α-lipoic acid/dihydrolipoic acid couple able to prevent hazardous reperfusion-induced alterations of heart mitochondria involved in the establishment of oxidative stress and energy depletion?

3. What is the mechanism by which DHLA/LA may possibly protect the heart from ischemia/reperfusion injury?

Abbreviations: DHLA, dihydrolipoic acid; LA, α-lipoic acid; $O_2^{\cdot-}$, superoxide radical; BSA, bovine serum albumin; RHM, rat heart mitochondria; P/O, ATP/oxygen ratio; RC, respiratory control index; UQ_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; UQ, ubiquinone; UQH_2, ubiquinol; $SQ^{\cdot-}$, ubisemiquinone radical.

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2. Material and methods

2.1. Chemicals

Bovine serum albumin (fraction V, BSA), fumaric acid, xanthine, xanthine oxidase, L-epinephrine, 5,5′-dithiobis-(2-nitrobenzoic acid) and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (UQ_0) were purchased from Sigma Chemical (St. Louis, USA). NADH and ADP were from Boehringer (Mannheim, Germany), triethanolamine was obtained from Fluka (Buchs, Switzerland). Dihydrolipoic acid and α-lipoic acid were a gift from ASTA Medica AG (Frankfurt, Germany). All other chemicals and biochemicals were purchased from Merck (Darmstadt, Germany).

2.2. Heart perfusion

Male Sprague-Dawley rats (300–350 g) were anaesthetized with ether and heparinized intravenously with 500 U/kg body weight. The hearts were rapidly removed and plunged into ice-cold perfusion medium until contractions have ceased. The heart was then cannulated via the aorta and perfused in the Langendorff mode [10] at a constant perfusion pressure of 70 mm Hg (9.33 kPa) at 37° C in a perfusion apparatus obtained from Hugo Sachs Electronic (March, Germany). After cannulation of the aorta, an incision was made in the pulmonary artery to ensure unhindered ejection of the coronary effluent. The perfusion medium was a Krebs-Henseleit buffer consisting of 118 mM NaCl, 5.8 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4 and 11 mM glucose. Before use, all solutions were filtered through a 0.2-μm-pore filter to remove any particulate contaminants. The perfusate was equilibrated with a 95% O_2/5% CO_2 gas mixture to adjust pH 7.4 at 37° C.

2.3. Functional parameters, ischemia, reperfusion

To measure the hemodynamic heart parameters a latex balloon was inserted into the left ventricle and inflated to a preload of 0.4 kPa. The left ventricular developed pressure, enddiastolic pressure, contractility and relaxation parameters were registered during the hole experiment with a ISOTEC pressure transducer connected to a Hugo Sachs Electronic PLUGSYS unit (March, Germany) and recorded on a multi-channel electrocardiograph (Zwönitz, Germany). Coronary flow was determined with a precalibrated flow meter from Hugo Sachs Electronic. The epicardial electrogram was registered in order to determine heart rate, duration and type of arrhythmias such as ventricular tachycardia and fibrillation. The definition of arrhythmias was performed according to the Lambeth conventions [11]. Rate pressure product was calculated out of the heart rate and the developed pressure. For the estimation of the procentual recovery of the hemodynamic parameters the preischemic control values were set as 100% and the parameters at the end of the reperfusion period were related to this value.

After a washout and equilibration period of 15 min hearts were perfused for 20 min to measure the preischemic control values. Then the hearts were subjected to 30 min of total global ischemia (37° C) by closing the aortic influx and then reperfused for 30 min by restorating the flow again. Control group was perfused with Krebs-Henseleit medium as usual while test groups were perfused with perfusion medium containing different concentrations of α-lipoic acid 15 min prior to ischemia and during reperfusion.

2.4. Heart mitochondria

Rat heart mitochondria (RHM) were isolated as described in [12]. The isolation buffer contained 0.3 M sucrose, 20 mM triethanolamine and 1 mM EDTA (pH 7.4). ATP/oxygen ratios (P/O) and respiratory control values (RC) were determined in the isolation buffer supplemented with 0.5 g BSA/l at 25° C with a micro Clark-type electrode of own design. Mitochondrial protein content was measured by the biuret method.

State IV respiration conditions were induced by the addition of 5 mM glutamate plus 5 mM malate. The transition to state III respiration was induced by the addition of 208 μM ADP and 2.5 mM inorganic phosphate.

2.5. Superoxide radical ($O_2^{\cdot-}$) formation

The $O_2^{\cdot-}$ production was measured with a Shimadzu 3000 UV-VIS spectrometer in a dual wavelength mode (480 nm/575 nm) using the SOD-sensitive epinephrine co-oxidation to adrenochrome. The reaction buffer consisted of 125 mM KCl, 2 mM EDTA, 20 mM triethanolamine, 725 U catalase/ml and 0.5 mM epinephrine and was saturated with pure oxygen. As a standard $O_2^{\cdot-}$ generating system 1 mM xanthine plus 3.3 μg/ml xanthine oxidase were used.

2.6. ESR measurements

Ubisemiquinone ($SQ^{\cdot-}$) populations in RHM established under control and 'ischemia' conditions were determined from ESR spectra of these compounds, using a Bruker ER 200 D-SRC spectrometer. 100 μl of RHM were preincubated with NADH in the presence and absence of DHLA. The suspension was equilibrated with 100% O_2 or 100% N_2, respectively, in order to establish aerobic or anaerobic conditions. The final volume (200 μl) was adjusted by the addition of adequate amounts of the reaction buffer. Respiration was initiated upon the addition of 20 mM succinate plus 4 mM fumarate. In the case of 'ischemia' (100% N_2) $K_3[Fe(CN)_6]$ (20 mM) was added to the suspension as an artificial e^- acceptor to keep the ubiquinone cycle running. The final suspension was filled

up into an ESR quartz tube (diameter: 3 mm) and immediately frozen in liquid nitrogen. ESR measurements were carried out at 200 K using a flow dewar and a temperature control unit. The spectrometer settings were: microwave frequency 9.43 GHz, power 1 mW, modulation frequency 100 kHz, modulation amplitude 4 G, receiver gain $4 \cdot 10^5$, points 1024, time constant 0.655 s, scans 4, sweep 80 G, centre field 3360 G, scan rate 57.2 G/min.

3. Results

In order to study the effect of α-lipoic acid on the myocardial reperfusion injury, isolated Langendorff perfused rat hearts were subjected to ischemia and reperfusion in the presence and absence of LA. Pretreatment of isolated hearts with 0.5 μM LA improved significantly the postischemic recovery of left ventricular developed pressure (Fig. 1C), contractility (Fig. 1D), relaxation (Fig. 1F) and the cardiac work parameters (Fig. 1B). The protective effect of LA was concentration-dependent. The optimal recovery was obtained with LA values around 0.5 μM in the perfusion medium while higher LA concentrations caused more intensive reperfusion injuries as seen in the controls (= postischemic reperfusion without LA). Electrophysiological parameters such as the duration of sinus rhythm (Fig. 2A) and ventricular fibrillation (Fig. 2B) remained unchanged in the presence of LA concentrations otherwise found to improve hemodynamic functions. Higher LA concentrations even impaired these electrophysiological activities exhibiting a similar response as shown before with respect to hemodynamic parameters (Fig. 1 and 2).

Since LA is metabolically reduced in the tissue to

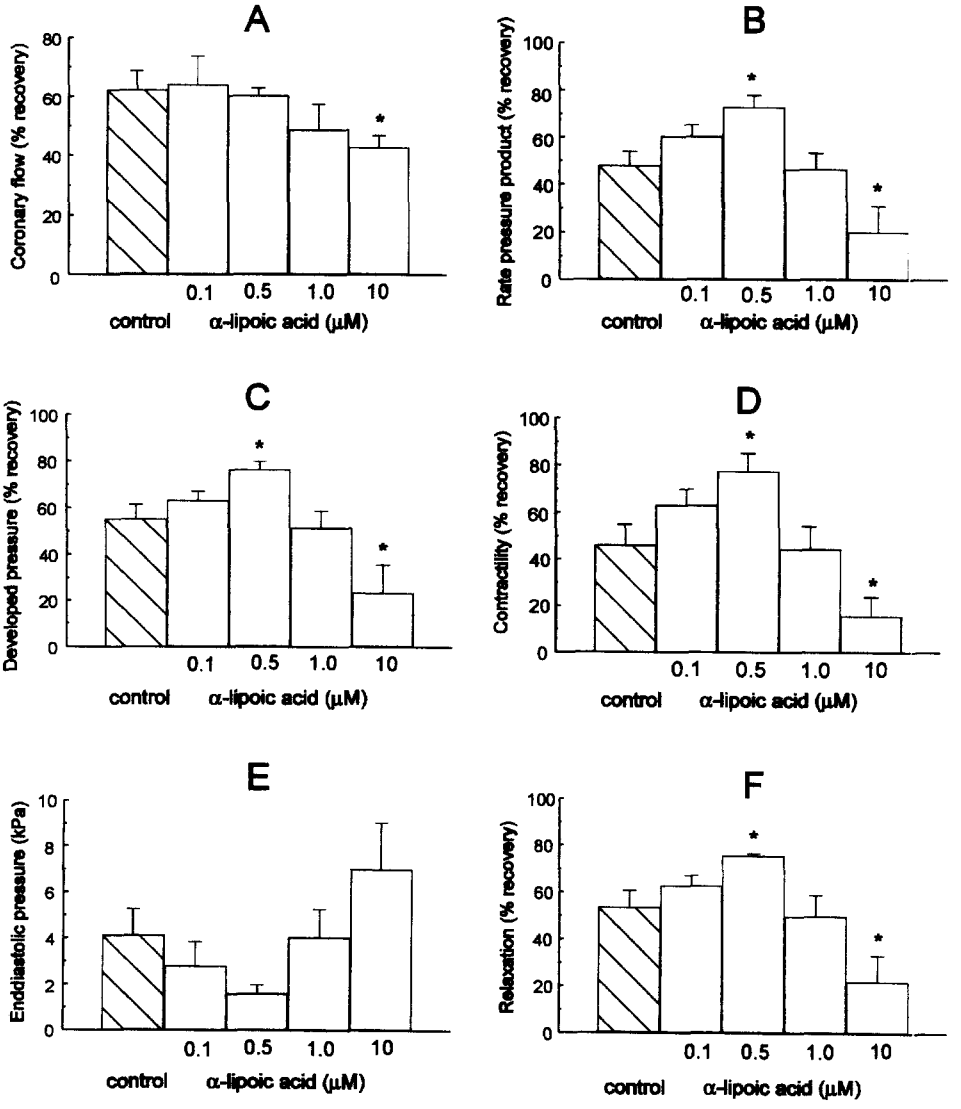

Fig. 1. Postischemic recovery of hemodynamic parameters of isolated perfused rat hearts subjected to 30 min of total global ischemia treated with different concentrations of α-lipoic acid before ischemia (15 min application) and during reperfusion (30 min). Data are mean ± S.E. ($n = 4$–6). * $P < 0.05$ vs. control group.

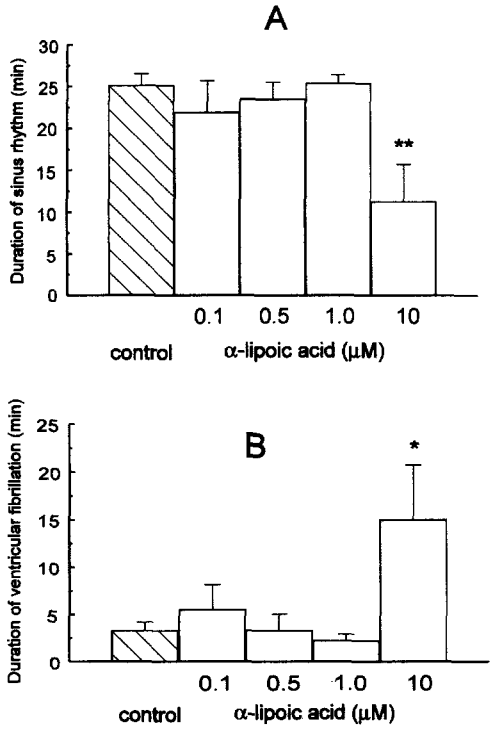

Fig. 2. Duration of sinus rhythm (A) and ventricular fibrillation (B) during 30 min of postischemic reperfusion in isolated rat hearts perfused pre- and postischemically with different concentrations of α-lipoic acid. Data are mean ± S.E. of 4–6 experiments. * $P < 0.05$, ** $P < 0.01$ vs. control hearts.

DHLA [13] all experiments with mitochondria were performed with DHLA. Mitochondria are equipped with the respective set of enzymes which establish an equilibrium between the oxidized and reduced form [9]. Thus, LA administered to the perfusion medium is readily transformed to DHLA in mitochondria.

To evaluate whether the protective effect of LA (DHLA) on hemodynamic heart parameters was related to a suppression of prooxidant induced heart injury we studied whether DHLA will interact with $O_2^{\cdot-}$-radical release from heart mitochondria. Heart mitochondria are unequivocally considered to be the major $O_2^{\cdot-}$ source responsible for the establishment of oxidative stress following reperfusion of ischemic hearts [14,15]. Isolation of mitochondria from an ischemic/reperfused tissue causes many artifacts. Therefore, a model system was used where the reperfusion injury was simulated by an exposure of isolated RHM to conditions normally developed in the tissue during ischemia/reperfusion. Recent studies revealed that among the various metabolic changes increased NADH levels (caused by anaerobic glycolysis) play a major role in the onset of oxidative stress [14]. Heart mitochondria were shown to have an organo-specific exogenous NADH dehydrogenase [16]. Activation of this mitochondrial enzyme over normal is responsible of functional changes of mitochondria during ischemia/reperfusion [14]. Apart from $O_2^{\cdot-}$ release which accompanies mitochondrial respiration activation of the exogenous NADH dehydrogenase by high NADH concentrations also impaired respiratory control values (Fig. 3A) and decreased ATP production with complex I substrates (Fig. 3B). Incubation of RHM with DHLA (from 1 to 40 nmol DHLA/mg protein) could not prevent the impairment of these respiratory parameters caused by NADH pretreatment.

However, DHLA caused a remarkable decrease of mitochondrial $O_2^{\cdot-}$ production. Fig. 4A shows that preincubation of RHM with NADH induced a significantly higher $O_2^{\cdot-}$ production rate (with succinate as respiratory substrate) as compared to untreated control RHM under otherwise identical conditions. When RHM were pretreated with DHLA before NADH exposure a dose-dependent reduction of $O_2^{\cdot-}$ generation was observed. In order to study whether the reduction of mitochondrial $O_2^{\cdot-}$ radical release was due to $O_2^{\cdot-}$ scavenging activities of DHLA the effect of the latter compound on the $O_2^{\cdot-}$ generating xanthine/xanthine oxidase couple was studied. $O_2^{\cdot-}$ formation rates from this biochemical source were adjusted

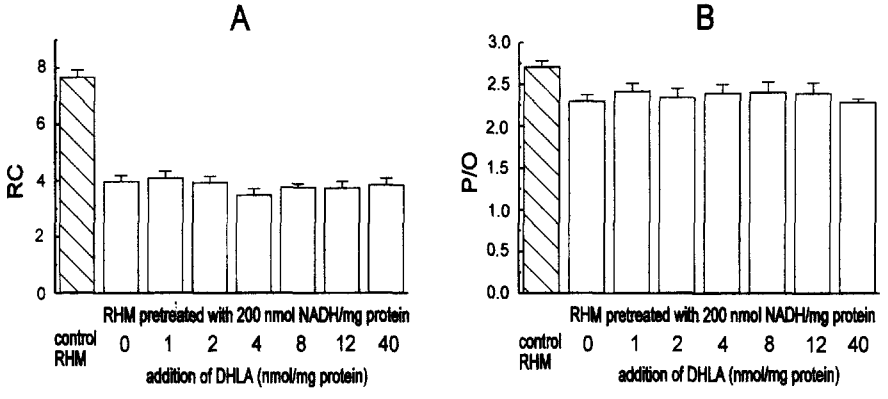

Fig. 3. Influence of dihydrolipoic acid on mitochondrial respiration parameters. Isolated RHM were exposed (in the reaction vessel, 0.3–0.4 mg protein/ml) to different concentrations of DHLA 1 min before addition of NADH. After complete exhaustion of NADH RHM were supplemented with inorganic phosphate and glutamate/malate (state IV). ATP generation was started following the addition of ADP. Data are mean ± S.E. of 3–9 experiments.

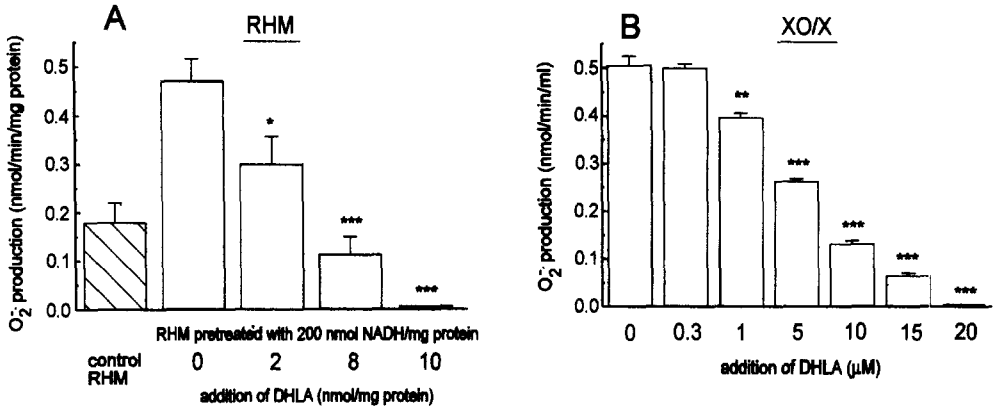

Fig. 4. Influence of dihydrolipoic acid on $O_2^{\cdot-}$ production (A) in RHM preincubated with different DHLA concentrations (at 4° C, 10 min) before exposure to NADH (200 nmol/mg protein, 20 min, at 4° C); $O_2^{\cdot-}$ production was measured under state IV respiration with 12 mM succinate; (B) in the xanthine oxidase/xanthine system adjusted such that $O_2^{\cdot-}$ production rates were similar to those obtained with isolated RHM. Data are mean ± S.E. of 3–4 experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. DHLA free group.

quantitatively to $O_2^{\cdot-}$ rates released from NADH-pretreated RHM (Fig. 4A, B). According to our observation at NADH-treated RHM the existence of free $O_2^{\cdot-}$ could be totally suppressed in the presence of sufficient amounts of DHLA.

A DHLA-induced inhibition of xanthine oxidase activities could be ruled out as a cause for the lack of detectable amounts of $O_2^{\cdot-}$ radicals. This became evident from the uninhibited formation of urate which also evolves from xanthine oxidase-catalyzed oxidation of xanthine (exp. not shown). Comparison of DHLA effects on the mitochondrial and the xanthine/xanthine oxidase system revealed that the total suppression of $O_2^{\cdot-}$ formation was twice as effective in RHM as in the xanthine/xanthine oxidase couple. This becomes visible when $O_2^{\cdot-}$ formation rates were plotted versus DHLA concentrations (Fig. 5).

Therefore, an additional mechanism affecting the mitochondrial $O_2^{\cdot-}$ release had to be taken into consideration. Due to the capability of DHLA to penetrate into phospholipid membranes a possible interference with the radical generating machinery of redox-cycling ubisemiquinone must also be considered as possible mechanism to reduce $O_2^{\cdot-}$ release. An effect of membrane-associated DHLA on mitochondrial steady state concentration of $SQ^{\cdot-}$ became evident from ESR experiments in which $SQ^{\cdot-}$-related ESR signals were followed in the absence and presence of DHLA (Fig. 6). DHLA was found to increase mitochondrial $SQ^{\cdot-}$ populations (76.0% vs. 64.4%; $P < 0.01$) in $O_2^{\cdot-}$-generating mitochondria (NADH pretreatment). Under anaerobic conditions (100% N_2 instead of 100% O_2) DHLA was without effect on mitochondrial $SQ^{\cdot-}$.

More detailed studies on this phenomenon were performed in a biochemical system in which UQ_0 was dissolved in water-free acetonitrile in the presence and absence of DHLA. Interestingly the presence of DHLA (but not of LA) was sufficient to generate $SQ^{\cdot-}$ radicals (Fig. 7B). The reaction was associated with a consumption of

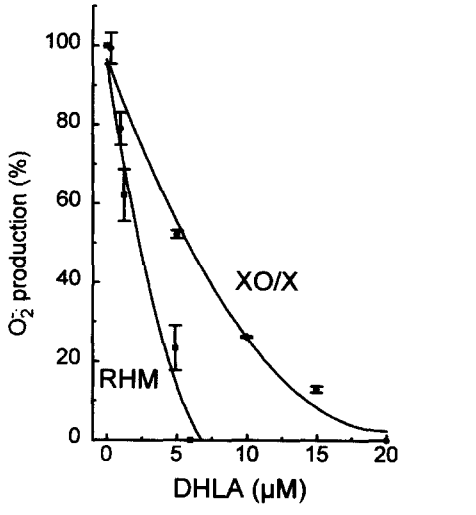

Fig. 5. Comparison between the influence of DHLA on the mitochondrial $O_2^{\cdot-}$ production and $O_2^{\cdot-}$ formation in the xanthine oxidase/xanthine system. $O_2^{\cdot-}$ production without DHLA was set as 100%. Experimental conditions: see Fig. 4.

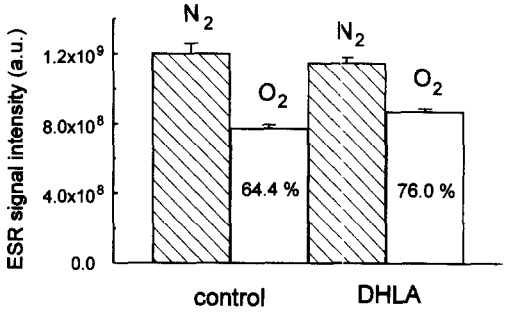

Fig. 6. Influence of DHLA (40 nmol/mg protein) on the ESR signal intensity of the ubisemiquinone radical in RHM. RHM were first exposed to NADH (10 mM, 25 min, at 4° C), for 10 min equilibrated with 100% O_2 or 100% N_2 and then supplemented with succinate/fumarate as substrates (in the case of N_2 $K_3[Fe(CN)_6]$ was added as electron acceptor). Data are mean ± S.E. of 5 experiments. ESR signal intensity of anaerobic RHM was set as 100%.

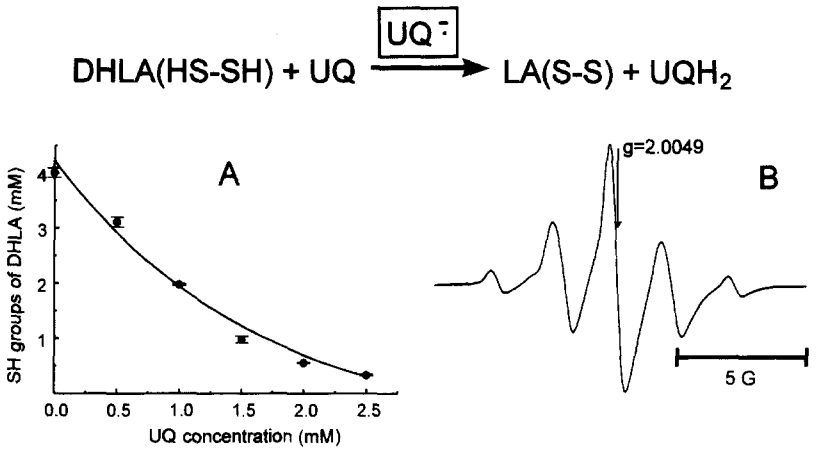

Fig. 7. (A) Oxidation of DHLA measured by means of decreasing SH group concentrations during the reaction of 2 mM DHLA with different amounts of UQ_0. SH groups were determined according to Ellman [17]. (B) Generation of a semiquinone radical in the reaction of DHLA with UQ_0. 1.22 mM UQ_0 was incubated with 0.61 mM DHLA in oxygen-free acetonitrile (dried with KOH). Stopped flow experiments were carried out at 25° C in a standard quartz flat cell. ESR spectrometer settings were: microwave frequency 9.74 GHz, power 5 mW, modulation frequency 100 kHz, modulation amplitude 1 G, receiver gain $2 \cdot 10^5$, time constant 0.3 s, scans 7, sweep 20 G, centre field 3482 G, sweep time 20.97 s.

SH groups as demonstrated by the dithiobis method (Fig. 7A). The disappearance of SH groups of the dithiol compound DHLA was directly related to the amount of UQ_0 present.

4. Discussion

Our studies support the existence of conflicting effects of DHLA/LA on the myocardial ischemia/reperfusion injury elsewhere reported in the literature. While some authors have reported on a protective action of LA or DHLA [13,18] others have found no effect of DHLA on the postischemic reperfusion injury [19]. The present investigation demonstrates that the effect of LA on physiological heart parameters was strongly dependent on the amount of LA inserted. With the exception of the coronary flow which was not affected all the other hemodynamic parameters were improved in the range of 0.5 μM LA. Higher LA concentrations (10 μM) intensified reperfusion injuries both on hemodynamic and electrophysiological heart functions. In contrast to the positive recovery effects of low LA concentrations on hemodynamic activities of reperfused hearts electrophysiological parameters showed no response but deterioration effects were developed as well at LA concentrations harmful to hemodynamic activities. The detrimental effect of higher LA concentrations which affected all physiological heart functions suggests that beneficial and harmful activities of LA are caused by different mechanisms.

The LA-related improvement of hemodynamic heart parameters cannot be explained on the basis of a more efficient energy supply in the form of ATP. In contrast to Zimmer et al. [8] we were unable to demonstrate any effect of LA (exp. not shown) or DHLA on mitochondrial energy-linked respiration. The well known impairment of mitochondrial oxidative phosphorylation during ischemia/reperfusion [14,18,20] was not improved. Zimmer et al. have drawn their conclusions from ATP synthase and ATPase studies of isolated RHM and mitoplasts. In their hands ATP synthase activities of mitoplasts accounted for only 20% of oxidative phosphorylation rates of intact mitochondria reported elsewhere by the same group [21]. Thus, it cannot be excluded that the effect of DHLA was studied at artificially altered mitochondrial preparations.

Since mitochondrial $O_2^{\cdot-}$ release was efficiently suppressed by DHLA one may conclude (i) the impairment of hemodynamic activities of reperfused hearts is a consequence of oxidative stress associated with ischemia/reperfusion and (ii) mitochondria represent the main site in the tissue where oxidative stress is established. In general radical scavenging activities of DHLA will also control radicals released from other sources. However, mitochondria are unequivocally accepted to play a major role in the establishment of oxidative stress [14,15] and DHLA was shown to affect both the formation and the existence of $O_2^{\cdot-}$ radicals from mitochondria. Apart from direct radical scavenging activity of DHLA which is analogue to scavenging reactions of other thiols DHLA [6,22–24] DHLA exerts an indirect radical scavenging effect which involves ubiquinol of mitochondria. As shown in our study DHLA together with UQ_0 gives rise to the formation of $SQ^{\cdot-}$ radicals in acetonitrile or increases the $SQ^{\cdot-}$ pool in respiring mitochondria. Oxidation of DHLA which was evaluated from the decrease of SH groups clearly indicated that reducing equivalents can be transferred from DHLA to UQ_0 (reaction 1). This observation is supported by reports in the literature demonstrating a stimulation of redox-cycling of various quinones when dihydrolipoamide was present [25]. The detection of $SQ^{\cdot-}$ in our in vitro system suggests the establishment of an equilibrium of all redox

states of UQ including UQH_2. The latter may result from a stepwise transfer of two e^- and protons from DHLA or the disproportionation reaction of two $SQ^{\cdot -}$ and the subsequent addition of protons from oxidized DHLA (reaction 2). In mitochondria an effect of DHLA on $SQ^{\cdot -}$ pools was only visible in the presence of oxygen. As earlier shown under our experimental conditions (NADH pretreatment as a model system for ischemia) redox-cycling $SQ^{\cdot -}$ is becoming autoxidizable ([26], reaction 3) which causes a decrease of $SQ^{\cdot -}$ pools (Fig. 6). Pretreatment of mitochondria with DHLA mitigates this effect such that $SQ^{\cdot -}$ pools increase (Fig. 6). According to Ref. [25] we propose the existence of a radical reaction between $O_2^{\cdot -}$ and UQH_2 which gives rise to the formation of $SQ^{\cdot -}$ and H_2O_2 while $O_2^{\cdot -}$ radicals disappear (reaction 4). We have work in progress demonstrating the existence of this type of reaction. Such a reaction can be expected to be highly efficient since due to the portion of DHLA (partition coefficient $P = 257$ [24]) which favours its accumulation in the lipid bilayer of the inner mitochondrial membrane DHLA is present in high amounts at the site where $O_2^{\cdot -}$ are formed. In contrast to the xanthine/xanthine oxidase system where $O_2^{\cdot -}$ can only be dissipated by a direct scavenging effect of DHLA mitochondria provide a further potential lipophilic $O_2^{\cdot -}$ scavenger namely UQH_2 which can be readily recycled by DHLA (according to reaction 1).

$$DHLA(HS-SH) + UQ \rightarrow LA(S-S) + UQH_2 \quad (1)$$

$$UQH_2 + UQ \rightleftarrows 2SQ^{\cdot -} + 2H^+ \quad (2)$$

$$SQ^{\cdot -} + O_2 \overset{(H^+)}{\rightleftarrows} UQ + O_2^{\cdot -} \quad (3)$$

$$UQH_2 + O_2^{\cdot -} \rightarrow SQ^{\cdot -} + H_2O_2 \quad (4)$$

Increasing LA concentrations were found to reduce all beneficial effects on the postischemic recovery of heart functions even causing deterioration of functional parameters at higher concentrations. From the literature on the metabolism of LA administered it is obvious that both the beneficial effects of low and the deteriorative effects of higher LA concentrations result from DHLA which is readily formed from LA in the tissue [9,13]. We believe that the antioxidative activity of DHLA is limited by the accumulation capacity of the membrane so that higher concentrations will lead to an enrichment of DHLA in the aqueous phase allowing stimulation of prooxidant activities. Evidence of the role of this dithiol compound in oxygen activation was demonstrated by an enhancement of oxidative degradation of desoxyribose [27] and ascorbic acid/iron-induced lipid peroxidation [28]. In the presence of transition metals hydroxyl radical formation occurs when DHLA is added as a reductant [27,29]. Prooxidant effects at concentrations around 0.5 mM were also found to exist with other thiol-containing compounds [29]. Decompartmentation and release of iron was recently reported to occur as a consequence of postischemic heart perfusion [30,31]. Functionally non-bound DHLA may supply reducing equivalents to ferric iron thereby increasing decompartmentation and running a Fenton-type reaction. Furthermore, DHLA when reacting with hydroxyl radicals may be transformed to thiyl or sulfonyl peroxyl radicals [32] which in turn may perpetuate peroxidative damage.

Our data have clearly demonstrated that LA (in its reduced form) mitigates postischemic hemodynamic impairments; furthermore, the antioxidant activity of low DHLA doses reveals the involvement of oxidative stress in the injury of mechanical heart functions. The concentration-dependent transition from an efficient antioxidant to a stimulator of oxidative stress can only be estimated quantitatively. Our study shows that in isolated perfused hearts a 10-fold increase of DHLA concentration required to obtain optimal protecting effects was detrimental.

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