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Original article

Opposite and tissue-specific effects of coenzyme Q_2 on mPTP opening and ROS production between heart and liver mitochondria: Role of complex I

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ABSTRACT

Coenzyme Q_2 (CoQ_2) is known to inhibit mitochondrial permeability transition pore (mPTP) opening in isolated rat liver mitochondria. In this study, we investigated and compared the effects of CoQ_2 on mPTP opening and ROS production in isolated rabbit heart and rat liver mitochondria. Mitochondria were isolated from New Zealand White rabbit hearts and Wistar rat livers. Oxygen consumption, Ca^{2+}-induced mPTP opening, ROS production and NADH DUb-reductase activity were measured. Rotenone was used to investigate the effect of CoQ_2 on respiratory complex I activity. CoQ_2 (23 μM) reduced the respiratory control index by 32% and 57% ($p<0.01$) in heart and liver mitochondria respectively, mainly through an increased oxygen consumption in state 4. CoQ_2 induced a 60% ($p<0.05$) decrease of calcium retention capacity (CRC) in heart mitochondria and inversely a 46% ($p<0.05$) increase in liver mitochondria. In basal condition, CoQ_2 induced a 170% ($p<0.05$) increase of H_2O_2 production in heart mitochondria and 21% (ns) decrease of H_2O_2 production in liver mitochondria. Because rotenone, a complex I inhibitor, increases H_2O_2 production in heart but not in liver mitochondria we investigated the CoQ_2 effect in a dose–response assay of complex I inhibition by rotenone in both mitochondria. CoQ_2 antagonized the effect of rotenone on respiratory complex I activity in liver but not in heart mitochondria. CoQ_2 significantly reduced NADH DUb-reductase activity in liver (−47%) and heart (−37%) mitochondria. In conclusion, our data showed that on the contrary to what was observed in liver mitochondria, CoQ_2 favors mPTP opening and ROS production in heart mitochondria through an opposite effect on respiratory complex I activity.

1. Introduction

Coenzyme Q (CoQ) is found in many intracellular organelles. CoQ chemical structure varies among species and tissues regarding the number of monounsaturated *trans*-isoprenoid units. CoQ_2 (Ub_{10}) chemical structure is constituted by 2 isoprenoid units. In mitochondria, CoQ is not only a component of the respiratory electron transport chain (ETC), but also presents several other physiological or pathophysiological functions [1,2].

Mitochondrial permeability transition pore (mPTP) opening has been shown to be a pivotal event in cell death. Selective inhibition of mPTP opening appears to be a promising therapeutic strategy against myocardial ischemia-reperfusion injuries [3,4]. Fontaine et al. showed that CoQ_2 inhibited mPTP opening in liver mitochondria; but did not affect mPTP opening in cultured liver Clone-9 and cancerous rat liver MH1C1 cells [5–7]. mPTP opening is regulated by several factors including Ca^{2+} overload, mitochondrial matrix pH, decreases of membrane potential, ATP, and excessive production of reactive oxygen species (ROS) [8–12]. Recent data suggested the implication of complex I in mPTP regulation [5].

The ETC significantly contributes to the production of intracellular ROS that are mainly localized in respiratory complexes I and III [13,14]. ROS were traditionally considered to cause damages to mitochondrial proteins, lipids, DNA and cell membranes. Recent studies have shown that a small amount of ROS acted as a sensor to stimulate signal transduction pathways feeding back to protect cells against lethal ischemia-reperfusion injuries [15–17].

In this study, we compared the effects of CoQ_2 on mPTP opening in isolated rabbit heart and rat liver mitochondria. We found opposite results between rabbit heart and rat liver mitochondria with CoQ_2 activating mPTP opening and increasing ROS production in isolated rabbit heart mitochondria. We also demonstrated that CoQ_2 interacts

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with respiratory complex I activity in rat liver mitochondria but not in rabbit heart mitochondria. Our results suggest that CoQ_2 effect on mPTP is tissue specific and may be related to its capacity to bind to the rotenone site in mitochondrial respiratory complex I.

2. Materials and methods

Animal experiments were performed in conformity with the *Guide for the Care and Use of Laboratory Animal* published by the US National Institute of Health (NIH Publication No. 85-23 revised 1996). All experimental protocols were carried out in accordance with the French (87/848, Ministry of agriculture and forestry) and European Community guidelines (86609/EEC).

2.1. Materials

All chemical reagents of the highest purity were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Calcium Green®-5N and Amplex Red® reagents were purchased from Molecular Probes, Inc. (Cergy Pontoise, France). CoQ_2 (Ub_{10}) were dissolved in absolute ethanol (0.5 mg/ml).

2.2. Animal preparation and isolation of mitochondria

Male New Zealand White rabbits (2.2 to 2.5 kg) were anesthetized with xylazine (5 mg/kg i.m.) and ketamine (50 mg/kg i.m.), and ventilated with room air. A catheter was inserted into the left carotid artery for continuous blood pressure monitoring. Limb lead II of the ECG was used for continuous heart rate monitoring. After the left thoracotomy was performed, a 20 min stabilization period was observed. At the end of this period, the left ventricle was harvested as previously described [18]. Male Wistar rat livers were harvested using the same anesthetic protocol. Mitochondria from heart or liver were isolated as previously described [19], resuspended in medium (pH 7.4) containing sucrose 70 mM and mannitol 210 mM in Tris 50 mM, at 4 °C. Mitochondrial protein concentration was determined by Biuret's method, using bovine serum albumin as standard [20]. Using this method, we obtained 16–18 mg of mitochondrial protein/g of tissue.

2.3. Measurement of mitochondrial calcium retention capacity (CRC)

CRC is defined here as the amount of calcium required to trigger mPTP opening *in vitro*, as previously described [21]. Measurement of CRC was performed at 25 °C using a spectrofluorophotometer F-2500 digi lab Hitachi® equipped with magnetic stirring and thermostatic control. Extra-mitochondrial free Ca^{2+} was measured in the presence of Calcium Green®-5N (1 μM) with excitation and emission wavelengths set at 500 and 530 nm respectively. Mitochondria (400 μg prot/2 ml) were resuspended in buffer (pH 7.4) containing 50 mM KCl, 2 mM KH_2PO_4, 20 mM Tris, 150 mM sucrose and 5 mM succinate at 25 °C. Following a 1 min preincubation period, 10 nmol of $CaCl_2$ was added every minute. As previously reported, after sufficient $CaCl_2$ loading, a rapid increase of fluorescence indicates a massive release of Ca^{2+} by mitochondria due to mPTP opening [21]. In the first set of experiments, increasing concentration of CoQ_2 was used to investigate the dose–response effect on CRC: 5, 10, 23, and 46 μM. CoQ_2 at 23 μM was used for the following experiments as previously described [22]. CRC measurement was also performed in the presence of L-ascorbic acid (L-A) (150 μM) an antioxidant or Cyclosporin A (CsA, 1 μM) an inhibitor of mPTP opening through its action on Cyclophiline D (CypD) to investigate the participation of ROS production and CypD in the effect of CoQ_2 on CRC. CRC was expressed as nmol Ca^{2+}/mg of protein (prot).

2.4. Measurement of mitochondrial oxygen consumption

Mitochondrial oxygen consumption was measured using a Clark-type oxygen electrode at 25 °C. Mitochondria (400 μg prot/ml) were incubated in the respiration buffer (pH 7.4) containing 60 mM KCl, 150 mM sucrose, 20 mM Tris–HCl and 5 mM KH_2PO_4. Glutamate/malate (complex I substrates, 5 mM each) were used in the absence (control) or presence of CoQ_2. State 3 (ADP-stimulated induced by addition of 0.3 mM ADP) and state 4 (ADP-limited: without ADP) respiration were determined and expressed as nmolO_2/min/mg prot. The respiratory control index (RCI) was calculated as the ratio of state 3/state 4.

The effect of CoQ_2 on complex I inhibition by rotenone was investigated using increasing doses of rotenone (0–1000 nM) in heart and liver mitochondria.

In addition, heart and liver mitochondria were frozen two times at −80 °C before evaluating CoQ_2 (23 μM) effects on NADH decylubiquinone (DUb)-reductase activity (complex I) as previously described [23,24].

2.5. Measurement of mitochondrial H_2O_2 production

H_2O_2 quantification has been accepted as an indicator of mitochondrial ROS production. Superoxide radicals produced by ETC are immediately converted into H_2O_2 catalyzed by superoxide dismutase [25]. Because H_2O_2 diffuses across mitochondrial membrane it can be used as an indicator of mitochondrial ROS production [26–28]. The rate of mitochondrial H_2O_2 production was measured at 25 °C in the presence of Amplex Red® (10 μM) and horseradish peroxidase (0.6 units) using a spectrofluorophotometer F-2500 digi lab Hitachi® (excitation and emission wavelengths set at 530 and 590 nm respectively). The rate of mitochondrial H_2O_2 production was measured in basal condition and with CoQ_2. Isolated mitochondria (200 μg prot) were added to 2 ml buffer (pH 7.4) containing 250 mM sucrose, 1 mM EDTA, 0.15% BSA and 1 mM EGTA in 20 mM Tris/HCl.

H_2O_2 production was also measured after stimulation either by complex I substrates glutamate/malate (5 mM each) or complex II substrate succinate (3.75 mM) in the absence or presence of CoQ_2. We also measured H_2O_2 production in the presence of rotenone (1 μM).

To verify the origin of fluorescence in the medium, we added 450 U/ml of catalase. The fluorescence was attenuated by 90–95%. The calibration curve was obtained by adding a known amount of H_2O_2 to the assay medium. The assay was linear from 0 to 150 nM H_2O_2. Fluorescence variation in time was used to measure H_2O_2 production. The results were expressed as pmol H_2O_2/min/mg prot.

2.6. Statistical analysis

All results are expressed as mean ± standard error of mean (SEM). Multiple group comparisons were performed using one-way analysis of variance (Graph Pad InStat) followed by Tukey's post-hoc test. Statistical significance was defined at $p \leq 0.05$.

3. Results

3.1. Calcium retention capacity (CRC)

In rabbit heart mitochondria, CoQ_2 decreased CRC value with a dose–response manner (Fig. 1, *inset*). CRC value averaged 605 ± 59 nmol Ca^{2+}/mg prot in control (Fig. 1). CoQ_2 at 23 μM significantly reduced CRC value to 219 ± 28 nmol Ca^{2+}/mg prot ($p<0.05$ *vs* control). Inversely, CoQ_2 significantly inhibited mPTP opening in rat liver mitochondria: CRC value was increased to 204 ± 22 nmol Ca^{2+}/mg prot compared to 110 ± 26 nmol Ca^{2+}/mg prot in the control group ($p<0.05$).