The Activation of Coenzyme Q Biosynthesis and Decreasing the Sensitivity of the Mitochondrial Permeability Transition Pore Opening Provide Cardioprotection against Ischemia-Reperfusion in Old Rats

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Abstract: The changes in functioning of isolated by Lanhendorf heart of old rats induced by an activation of ubiquinone - coenzyme Q (CoQ) endogenous synthesis via administration of its precursors – 4-hydroxybenzoic acid, methionine and a modulator of vitamin E were studied. An activation of ubiquinone biosynthesis contributed to cardioprotection against the development of the heart postreperfusion injures and also to a decrease in the sensitivity of mitochondrial permeability transition pore opening to Ca2+ in the rat heart in aging

Key-Words: coenzyme Q biosynthesis, ischemia-reperfusion, mitochondrial permeability transition pore, heart, aging

1 INTRODUCTION

Ischemia-reperfusion is a very common cause of functional disorders of the heart resulting from an increase in availability of calcium ions, substrates for lipid peroxidation and free radicals of oxygen and nitrogen in cardiomyocytes. The mitochondrial permeability transition pore (MPTP) opening is known to contribute to dysfunction and injury during reperfusion of the ischemic heart. MPTP is a mitochondrial channel, its opening results in collapse of mitochondrial membrane potential which leads to apoptosis so inhibiting of MPTP may serve an important way of cardioprotection [4, 7, 8]. Antioxidant system ubiquinone - coenzyme Q (CoQ) was demonstrated to prevent oxidative stress in cardiomyocytes. CoQ is the key component of cellular bioenergetics as a transporter of protons and electrons in mitochondrial electron-transport chain, and it is an important lipid-soluble endogenously synthesized antioxidant [11, 1, 9]. The role of CoQ in regulation of MPTP, involved in apoptotic pathways, was also demonstrated [7]. Endogenous CoQ biosynthesis is a multi-step process with subtle regulation mechanisms that are often disrupted under numerous pathological conditions and even under irrational feeding, deficit of vitamins, negative environmental impact, and in aging. At present, possible ways of increasing CoQ availability in an organism have being studied [10]. Since an increase in intracellular CoQ pool might result from
either exogenic CoQ input or enhancement of its endogenous synthesis, and investigation of the mechanisms of an activation of CoQ endogenous synthesis is believed to be more important, the aim of the present work was to study the effects of precursors of CoQ biosynthesis on the functional state of isolated by Lanhendorf heart during ischemia-reperfusion, and to study the sensitivity of MPTP opening to Ca$^{2+}$ in old rats.

2 MATERIAL AND METHODS

Male Wistar rats aged 6 – 7 months with body mass of 200 – 250 g and male Wistar rats aged 24 months with body mass of 350 – 450 g were fed with standart food during the whole experiment. The precursors of CoQ biosynthesis – 4-hydroxybenzoic acid (100 mg/kg body weight) as a precursor of benzoquinone nucleus of CoQ and methionine (100 mg/kg body weight) as a donor of methyl radicals and α-tocopherol (10 mg/kg body weight) as a modulator of CoQ synthesis were administered per os daily for 10 days. The animals were divided into 3 groups each of 12 rats: the 1st group – control adult animals; the 2nd group – control old animals; the 3rd group – old animals treated with the precursors of CoQ biosynthesis. The experiments on animals were provided in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mitochondria were isolated from the heart by the method of differential ultracentrifugation [5]. In the heart homogenates and mitochondria, CoQ and vitamin E were separated by thin layer chromatography, and they were determined spectrophotometrically [2]. Myocardial function was evaluated using the Lanhendorf isolated rat hearts [3]. An isolated working heart preparations were exposed to 30 min of global ischaemia followed by 40 min reperfusion. We analyzed the data of myocardial contractile function, coronary flow and end diastolic pressure. Ca$^{2+}$ – induced MPTP opening was determined using spectrophotometric assay of mitochondrial swelling [8]. The results were processed with methods of variation statistics using Excel (MS Office XP) and Origin 6.0 (Origin Lab Corporation). The data were presented as an average with standard deviation (M±m). Differences were considered statistically significant when $p < 0.05$. Reliability of differences between two values was estimated with Student’s t-test.

3 RESULTS AND DISCUSSION

In our earlier research, we demonstrated that administration of the precursors of CoQ biosynthesis induced an increase in CoQ and vitamin E content in the rat heart mitochondria 1.5 and 1.8 times as such that control [6].

![Graph A](image1)

**Fig.1.** Effect of ischemia-reperfusion injury on the left ventricular developed pressure (A) and end diastolic pressure (B) in the heart of
old rats in the control (1) and after an activation of CoQ synthesis in rats (2) (n=7). * P<0,05 as compared with control

The data obtained in this work is evidence that an activation of CoQ biosynthesis contributes to cardioprotection by reducing the degree of the ischemia-reperfusion injury in the heart of old rats, resulting from the restoration of myocardial contractile function (Fig.1A), coronary flow (Fig.2), and a decrease in the end diastolic pressure (Fig.1B) of the heart compared with the control group of the animals during ischemia-reperfusion.

Fig.2. Effect of ischemia-reperfusion injury on the coronary flow in the heart of old rats in the control (1) and after an activation of CoQ synthesis in rats (2) (n=7). * P<0,05 as compared with control.

In our experiments, Ca\(^{2+}\) – induced MPTP opening was fully prevented by its classic inhibitor cyclosporine A (10\(^{-5}\) mol/l) which ascertains the fact that MPTP opening plays an important role in observed mitochondrial swelling. In old rats, an administration of precursors of CoQ biosynthesis led to more significant decrease in the sensitivity of MPTP opening to its inductor Ca\(^{2+}\) (10\(^{-4}\) mol/l) that in control (Fig.3).

Fig.3. Effect of activation of CoQ synthesis on swelling of mitochondria from the heart of old rats in a presence of Ca\(^{2+}\) inductor (10\(^{-4}\) mol/l) (n=5): 1 – heart mitochondria from adult rat; 2 – heart mitochondria from old rat; 3– heart mitochondria from old rat after administration of CoQ biosynthesis precursors; 4 – heart mitochondria from old rat in presence Ca\(^{2+}\); 5 – heart mitochondria from old rat after administration of CoQ biosynthesis precursors in the presence Ca\(^{2+}\). Thus, the results allow to conclude that activation of CoQ biosynthesis by administrating of its precursors exerts protective effect on the development of the heart postreperfusion injures and on the MPTP- opening in the heart with aging.

References


