Pharmacological Research 76 (2013) 17–27

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Rice bran extract protects from mitochondrial dysfunction in guinea pig brains

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ARTICLE INFO

Article history:
Received 11 April 2013
Received in revised form 13 June 2013
Accepted 18 June 2013

Keywords:
Bioenergetics
Mitochondria
Brain
Nutrition
Respiration
Cardiolipin
Mitochondrial dynamics
Nitrosative stress
Oxidative stress
Rice bran extract

ABSTRACT

Mitochondrial dysfunction plays a major role in the development of age-related neurodegenerative diseases and recent evidence suggests that food ingredients can improve mitochondrial function. In the current study we investigated the effects of feeding a stabilized rice bran extract (RBE) on mitochondrial function in the brain of guinea pigs. Key components of the rice bran are oryzanols, tocopherols and tocotrienols, which are supposed to have beneficial effects on mitochondrial function. Concentrations of α-tocotrienol and γ-carboxyethyl hydroxychroman (CEHC) but not γ-tocotrienol were significantly elevated in brains of RBE fed animals and thus may have provided protective properties. Overall respiration and mitochondrial coupling were significantly enhanced in isolated mitochondria, which suggests improved mitochondrial function in brains of RBE fed animals. Cells isolated from brains of RBE fed animals showed significantly higher mitochondrial membrane potential and ATP levels after sodium nitroprusside (SNP) challenge indicating resistance against mitochondrial dysfunction. Experimental evidence indicated increased mitochondrial mass in guinea pig brains, e.g. enhanced citrate synthase activity, increased cardiolipin as well as respiratory chain complex I and II and TIMM levels. In addition levels of Drp1 and fis1 were also increased in brains of guinea pigs fed RBE, indicating enhanced fission events. Thus, RBE represents a potential nutraceutical for the prevention of mitochondrial dysfunction and oxidative stress in brain aging and neurodegenerative diseases.

1. Introduction

Population aging is one of the major challenges for societies worldwide [1]. Aging is considered one major risk factor for neurodegenerative diseases such as Alzheimer's or Parkinson's disease [2,3]. Currently approved drugs only attenuate the symptoms, but do not cure these conditions. Therefore, new scientific approaches focus on strategies to avoid such maladies by long-term nutrition based intervention. Mitochondrial dysfunction seems to play a major role in the development of age-related neurodegenerative diseases [4–7] and recent evidence suggests that mitochondrial dysfunction can be prevented by food ingredients, including polyphenols, omega-3 fatty acids and vitamin E [8–11].

Mitochondria are complex, network forming organelles, involved in different metabolic pathways, e.g., citric acid cycle (TCA), energy transformation, amino acid metabolism and urea cycle [12]. Mitochondria consist of inner and outer membranes composed of phospholipid bilayers and proteins. The outer mitochondrial membrane, which encloses the entire organelle, contains numerous integral proteins. These porins are responsible for the high permeability of the outer membrane to all molecules up to 5000 daltons, whereas the inner mitochondrial membrane is quite tight. Mitochondrial membranes contain translocases for protein import [13] and various specific mitochondrial carriers in the inner membrane for the import of hydrophilic compounds [14]. The inner mitochondrial membrane harbors the proteins of the electron

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http://dx.doi.org/10.1016/j.phrs.2013.06.008

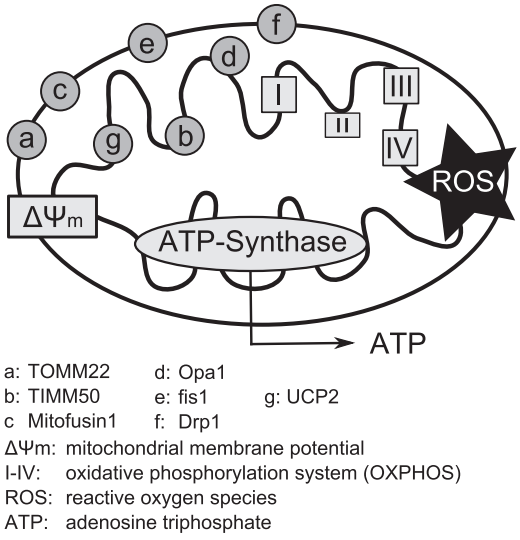

Fig. 1. Mitochondria are complex, network forming organelles that are crucial for the energy metabolism of the cell. Mitochondria consist of an outer membrane and an inner membrane that is intensely folded to yield a large membrane surface. The four complexes of the electron transport system are embedded in the inner mitochondrial membrane. While electrons from electron donors like NADH or succinate are transported along the complexes, complexes I, III and IV transport protons from the matrix to the intermembrane space and thus create a mitochondrial membrane potential ($\Delta\psi_m$). The mitochondrial membrane potential is used by complex V to produce ATP from ADP and inorganic phosphate. Failure in the electron transport system leads to the production of reactive oxygen species (ROS) like hydrogen peroxide that can damage macromolecules and thus lead to dysfunctional cell components or even apoptosis. Mitochondria constantly undergo fusion and fission to maintain appropriate function, disperse partially damaged mitochondria and create new organelles. Important proteins for fission and fusion are Mitofusin-1, optic atrophy 1 (Opa1), fission 1 (fis1) and dynamin-related protein 1 (Drp1) [46]. Translocase of the outer/inner mitochondrial membrane (TOMM/TIMM) are very important protein complexes that allow protein import into the intermembrane space (TOMM) and the matrix (TIMM).

transfer system (ETS), responsible for oxidative phosphorylation. The mitochondrial oxidative phosphorylation (OXPHOS) system is the final biochemical pathway producing energy in form of ATP by consuming oxygen. From complex I and II electrons are transferred to complex III by Coenzyme Q, the glycerophosphate dehydrogenase and the electron transferring flavoprotein. From complex III the electrons are transferred to oxygen via cytochrome *c* and complex IV. Simultaneously, an electrochemical proton gradient is build across the inner mitochondrial membrane (by complex I, III, and IV) and the generated protonmotive force is used by complex V to produce ATP (Fig. 1) [15,16].

Alterations of mitochondrial efficiency and function are mainly related to alterations in mitochondrial content, amount of respiratory enzymes or changes in enzyme activities [17–20]. Growing evidence indicates that changes of the network are related to bioenergetic function and the consequences are a matter of intensive research [21–23]. A reduction in mitochondrial content or lowered ETS results in a general limitation of energy production. Dysfunction of single complexes of the respiratory system are frequently accompanied by deleterious side effects like loss of mitochondrial membrane potential (MMP) and consequently decreased ATP levels, but also production of reactive oxygen species (ROS) [24].

Rice bran is produced as a by-product in the rice milling process, a method in which the outer layer of the rice grain is removed. Rice bran has various biological effects like anti-inflammatory, cholesterol-lowering, antioxidant and anti-diabetic activities [25]. Rice bran contains the enzyme lipase which quickly renders the bran rancid and inedible. The bran can be stabilized and thus preserved to allow usage as a food ingredient [26].

Key components of rice bran are oryzanols (a mixture of ferulic acid esters of triterpene alcohol and phytosterols), tocopherols and tocotrienols [25]. Compared to the pure bran these components are concentrated in the stabilized Egyptian rice bran extract (RBE) used in the current study. Thus, RBE rather provides pharmacological than nutritional effects and it is intended to be used as an oral antidiabetic in the near-East [27]. In the current study we assessed the neuroprotective potential of pharmacological doses of RBE rich in vitamin E and γ-oryzanol in healthy young guinea pigs. Tocotrienols and tocopherols are supposed to have beneficial effects on mitochondrial function [28,29] whereupon recent data suggest that tocotrienols are more effective mitochondrial protectors than tocopherols [29,30]. Therefore rice bran may be a suitable food to protect mitochondria and to improve mitochondrial dysfunction.

RBE was daily administered by oral gavage for 30 days and brain mitochondrial parameters and function were tested in isolated mitochondria as well as in brain cells.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, chemicals were of highest available purity and purchased from Sigma (Grand Island, NY, USA) or Merck (Darmstadt, Germany). Aqueous solutions were prepared with deionized, filtered water (Millipore, Billerica, MA, USA). Heat stabilized Egyptian rice bran extract was obtained from IT&M S.A. (Giza, Egypt, Supplementary Fig. 2). After overnight maceration in hexane three successive extraction sessions under reflux at 40 °C were applied. The extraction ratio was 3:1. The extract was evaporated under vacuum at a temperature not exceeding 50 °C.

2.2. PC12 cell culture

PC12 cells were maintained in DMEM medium supplemented (v/v) with 10% FBS, 5% HS and 1% antibiotics [31]. Cells were split twice per week. For the actual experiments, cells were cultured in 6-well plates. RBE stock solution was prepared in DMSO and vehicle concentrations were ≤0.5%. After being allowed to attach for about 24 h in DMEM, cells were exposed to RBE (0.3 mg/ml). After 24 h cells were harvested and snap-frozen for Western blot analysis.

2.3. Animals and treatment

Male Dunkin Hartley guinea pigs (300 g) were purchased from Charles River (Sulzbach, Germany) and randomly divided into 3 groups of 6 animals (control, RB50 and RB150). RBE was administered by oral gavage once a day for 30 days. The control group received an agarose solution (0.2%); RB50 received 50 mg RBE/kg body weight/day dissolved in agarose solution, RB150 received 150 mg RBE/kg body weight/day. Additionally, the animals had ad libitum access to drinking water and a standard pellet diet.

The protocol for the animal feeding study and tissue collection was approved by the local authorities for animal welfare and all experiments were carried out according to the European Communities Council Directive (86/609/EEC) by individuals with appropriate training. Guinea pigs were sacrificed by decapitation. Blood was centrifuged in heparinized vials at 3000 rpm. The obtained plasma samples were immediately frozen at −80 °C for further experiments. The brain was quickly dissected on ice after removal of the cerebellum and brain stem. Since tissue was used for biochemical analysis brains were not perfused before decapitation. However, based on an assumed average brain plasma volume of 10.3 μl/g after decapitation the influence of residual blood on the determined analytical parameters can be neglected [32].