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Uncouple my heart: the benefits of inefficiency

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Abstract Myocardial ischemia/reperfusion (IR) injury leads to structural changes in the heart muscle later followed by functional decline due to progressive fibrous replacement. Hence approaches to minimize IR injury are devised, including ischemic pre—and postconditioning. Mild uncoupling of oxidative phosphorylation is one of the mechanisms suggested to be cardioprotective as chemical uncoupling mimics ischemic preconditioning. Uncoupling protein 2 is proposed to be the physiological counterpart of chemical uncouplers and is thought to be a part of the protective machinery of cardiomyocytes. Morphological changes in the mitochondrial network likely accompany the uncoupling with mitochondrial fission dampening the signals leading to cardiomyocyte death. Here we review recent data on the role of uncoupling in cardioprotection and propose that low concentrations of dietary polyphenols may elicit the same cardioprotective effect as dinitrophenol and FCCP, perhaps accounting for the famed "French paradox".

Keywords Uncoupling protein 2 · Oxidative phosphorylation · Polyphenols · Ischemia pre-conditioning

Introduction

Mitochondria are irreplaceable source of ATP for the high energy demanding cardiac muscle cells who utilize it for incessant contraction/relaxation thus driving the heartbeat. Consequently, dysfunction of mitochondria results in either necrotic or apoptotic death of cardiomyocytes. Damage to mitochondria appears to be the result of ischemia reperfusion injury, i.e. restoration of oxygen flow after a period of ischemia, which is accompanied by increase in reactive oxygen species (ROS) level and intracellular calcium (Halestrap et al. 2007). Halestrap also points out that ischemia preconditioning (IP) reduces ROS levels during ischemia-reperfusion by evoking decreased ROS production or improved ROS removal. Because IP protects against oxidative stress mediated by exogenous source (Morihira et al. 2006), it appears that improved ROS removal is the agent of salvation.

ROS are formed during mitochondrial respiration with conversion of oxygen to superoxide anion ($^{\bullet}O_2^-$) occurring at Complex I and Complex III (Skulachev 1998; Pedersen 1999; Raha and Robinson 2000). Most ROS are formed in vivo under the non-phosphorylating, "resting", state of mitochondria. Increasing slightly mitochondrial respiration shortens lifetime of the ubisemiquinone anion radical ($UQ^{-\bullet}$) and leads to a lowered oxygen tension in the microenvironment. Besides natural cellular activity, such as contraction/relaxation, increased respiration may also stem from increase of the H^+ backflux into the matrix, which diminishes mitochondrial potential Δp and results in a decrease of mitochondrial ROS formation. The H^+ backflow may be a simple leak, i.e. change in membrane permeability due to structural reasons for example lipid composition, or a protein-mediated process (Korshunov et al. 1997). Chemical uncouplers, however, are known to elicit the same effect.

Ischemia preconditioning by chemical uncouplers

Chemical uncouplers provide an alternative entry pathway for protons ejected into the intermembrane space of

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mitochondria as described in the Mitchell chemiosmotic theory (Mitchell 1961). Known uncouplers are invariably weak acids capable of penetrating the membrane as neutral or charged species. Quantitative structure-activity relationships for uncoupling ability of weak acids are sought from the point of view of toxicity of these compounds (Spycher et al. 2008). Because uncoupling of oxidative phosphorylation predictably causes inefficient use of energy stores, fatty acids especially, development of pharmacological agents that help in caloric restriction is still actively sought. This is despite the negative side-effects of dinitrophenol (DNP) that occured when it was used as a diet pill in the 1930s (Blaikie et al. 2006; Caldeira da Silva et al. 2008).

Natural uncoupling activity leads to higher resting energy expenditure and appears to support longevity (Speakman et al. 2004). Inefficiency of oxidative phosphorylation elicited by uncouplers increases the idle turnover of respiration, like a car engine idling at a higher rpm after start-up, hence diminishing ROS formation (vide supra). Is it enough to protect the heart against IR injury? While the overall effect may not be attributable solely to down-regulation of ROS, uncouplers have been shown to protect isolated hearts against IR injury (Minners et al. 2000; Brennan et al. 2006a; Brennan et al. 2006b).

Mitochondria contain superoxide dismutase to curb possible damage evoked by superoxide formed, but the emerging hydrogen peroxide can diffuse across membranes and is freely distributed in the cell, where it may be metabolized into more reactive radicals via free transition metal-mediated Fenton reactions. Therefore, means to prevent the formation of the initial unwanted product, i.e. superoxide, are devised perhaps in the form of a protein activated by superoxide and activation of which delimits superoxide formation (Echtay et al. 2002).

Uncoupling protein 2 as a physiological cardioprotective unit

Uncoupling protein 2 (UcP2) is an inner mitochondrial membrane protein, a close homolog of the well-known UcP1, also called thermogenin, which is expressed exclusively in brown fat mitochondria. Tissue expression pattern of UcP2 is much broader including pancreatic β-cells, white fat, heart, brain, and macrophages. While discussions on physiological activity of UcP2 ensue, its general capability to mediate free fatty acid anion transport across membranes is now accepted. There is extensive evidence that UcP2 attenuates mitochondrial production of ROS (Brand and Esteves 2005). This attenuation, along with UcP2 up-regulation, is considered the basis of ischemia tolerance observed in cardiomyocytes subjected to ischemia preconditioning (McLeod et al. 2005). In rat neonatal cardiomyocytes, overexpression of UcP2 confers tolerance to oxidative stress via diminished mitochondrial Ca^{2+} overload and reduced generation of ROS (Teshima et al. 2003). Moreover, UcP2 may be involved directly in regulating mitochondrial Ca^{2+} uptake thereby facilitating responsiveness of a cell to calcium signalling (Graier et al. 2008). UcP2 has also been found cytoprotective in the brain, where it was shown to play a role in modulating neuronal tolerance to ischemic stress [reviewed in (Andrews et al. 2005)].

Mild uncoupling, likely performed by UcP2, is probably designed to limit ROS production by preventing large increase in proton gradient if ADP is not available (conditions of high ATP:ADP ratio). High levels of ROS were observed in macrophages of UcP2 -/- mice, which showed higher mitochondrial damage due to oxidative stress while possessing better antibacterial properties (Arsenijevic et al. 2000). Exposure to oxidative stress up-regulates UcP2 in clonal beta-cells (Li et al. 2001). This fits with observations of UcP2 down-regulation when oxidative stress is reduced.

Modulation of UcP2 level appears important because the protein increases in the failing human heart (Murray et al. 2004) and was shown to increase sensitivity of adult rat cardiomyocytes to IR injury (Bodyak et al. 2007). More-

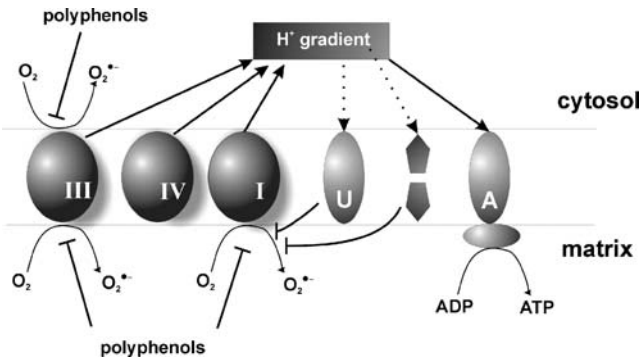

Fig. 1 Polyphenols as uncouplers of oxidative phosphorylation. Schematic depiction of the respiratory chain, from which complex II and cytochrome c are omitted for clarity, shows the formation of a proton gradient across the inner mitochondrial membrane. Complexes **I**, **III**, and **IV**, with the order shown in the figure being intentionally distorted, eject protons into the intermembrane space creating a proton gradient. From there protons normally re-enter the matrix through the ATP synthase (**A**) thus driving ATP synthesis. An alternative proton re-entry point is uncoupling protein 2 (**U**) that requires free fatty acids or lipoperoxidation products, e.g. 4-hydroxynonenal, to facilitate a proton leak. Other alternatives for proton re-entry into the matrix are chemical uncouplers, depicted as two facing pentagons, which include polyphenols. Uncoupling of oxidative phosphorylation diminishes superoxide formation by complex **I,** which is known to be uncoupler sensitive**.** In addition, polyphenols may inhibit superoxide formation by complexes **I** and **III** by virtue of their antioxidant ability

over, UcP2 is a reported candidate gene that correlates with the development of β-adrenergic stimulation induced cardiomyopathy (Gaussin et al. 2003). Investigations into regulation of UcP2 expression identified several effectors including thyroid hormones, TNFα, cAMP, and lipids, all of which may converge on e.g. p38-MAPK regulated pathway (Valouskova and Modriansky 2008). Antioxidants could facilitate down-regulation of UcP2 by limiting oxidative stress, but they actually may have the opposite effect, too. Tempol, a representative nitroxide and a potent antioxidant, increased UcP2 level in heart (Mitchell et al. 2003) while edaravone, a potent free radical scavenger, prevented isoproterenol-mediated up-regulation of UcP2 (Ishizawa et al. 2006).

Polyphenols: antioxidants and uncouplers

Polyphenols are a class of substances containing several hydroxyl groups on aromatic rings thus forming a very diverse group of compounds present in our diet. Certain polyphenols, e.g. quercetin, are found in all plant derived foods, whereas others are endemic. (for review cf. (Manach et al. 2004)). Diversity of polyphenols is behind the various biological activities but generally many polyphenols were found to possess antioxidant properties. Therefore a plausible hypothesis is put forth that the antioxidant activity along with capacity to penetrate membranes results in ROS scavenging within the mitochondrial matrix.

On the other hand, polyphenols are also weak acids and depending on their structure may fit the model and biological activity of a chemical uncoupler (Fig. 1). As an example, galangin, or 3,5,7-trihydroxyflavone, was shown to behave as an uncoupler (Dorta et al. 2008). Interestingly, lower concentration of galangin (10 μM) was more effective in preventing lipid peroxidation than higher concentration (50 μM) resembling effectiveness of FCCP in preventing IR injury (Brennan et al. 2006a). Indeed, cardioprotection seen with protonophores should oxidize mitochondria but should not depolarize them.

Besides possessing antioxidant and uncoupling abilities, polyphenols influence mitochondrial reticulum morphology. Resveratrol increases the number of mitochondria which may advance higher resistance of the mitochondrial reticulum towards damage stemming from IR injury (Baur et al. 2006). A single mitochondrion is observed in an intact cell, whereas stress causes fragmentation of mitochondria (Benard and Rossignol 2008). Bidirectional relationship between mitochondrial structure and energetics suggests that uncoupling of oxidative phosphorylation, perhaps evoked by continuous presence of low concentration of dietary polyphenols, affects ROS levels as well as mitochondrial morphology in a way favorable for cardiomyocyte survival of ischemia/reperfusion event.

Conclusion

Bioavailability of polyphenols is limited. Their detected plasma concentrations are below 1 μM, and their metabolites are mostly found in tested subjects (Manach and Donovan 2004). Polyphenols fit the profile of a multitarget drug suitable for the concept of polypharmacology or network pharmacology (Hopkins 2008), although the opportunity window may be narrow for polyphenols exhibiting uncoupling activity. Steady intake of polyphenols in the diet may, perhaps because of the limited bioavailability, facilitate the desired effect of mild uncoupling turning the mitochondrial inefficiency into an advantage and protect cells, in case of cardiomyocytes, against IR injury.

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