9L.3 Mitochondrial generated nitric oxide protects against permeability transition via formation of membrane protein S-nitrosothiols

Ana C.R. Leite1, Helena C.F. Oliveira1, Fabiane L. Urito2, Rafael García2, Luciane C. Alberici2, Mariana P. Fernandes2, Roger F. Castilho2, Anibal E. Vercesi2

1Department of Physiology and Biophysics, Institute of Biology, State University of Campinas, Brazil
2Department of Clinical Pathology, Faculty of Medical Sciences, State University of Campinas, Brazil
E-mail: anibal@unicamp.br

Mitochondria generated nitric oxide (NO) regulates several cell functions including energy metabolism, cell cycling, and cell death. Here we report that the NO synthase inhibitors (L-NAME, L-NNA and L-NMMA) administered either in vitro or in vivo induce Ca\(^{2+}\)-dependent mitochondrial permeability transition (MPT) in rat liver mitochondria via a mechanism independent on changes in the energy state of the organelle. MPT was determined by the occurrence of cyclosporin A sensitive mitochondrial membrane potential disruption followed by mitochondrial swelling and Ca\(^{2+}\) release. In in vitro experiments, the effect of NOS inhibitors was dose dependent (1 to 50 \(\mu\)M). In addition to cyclosporin A, L-NAME induced MPT was sensitive to Mg\(^{2+}\) plus ATP, EGTA, and to a lower degree, to catechol and dithiothreitol. In contrast to L-NAME, its isomer d-NAME did not induce MPT. L-NAME induced MPT was associated with a significant decrease in both the rate of NO generation and the content of membrane protein S-nitrosothiol. Acute and chronic in vivo treatments with L-NAME also promoted MPT and decreased the content of mitochondrial protein S-nitrosothiol. SNAP (a NO donor) prevented L-NAME mediated MPT and reversed the decrease in the rate of NO generation and in the content of membrane protein S-nitrosothiol. We propose that S-nitrosylation of critical membrane protein thiols by NO protects against MPT.

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Posters

9P.1 Stigmatellin as a modulator of metal-induced inner mitochondrial membrane permeabilization

Elena A. Belyaeva

Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Sciences, Laboratory of Comparative Biochemistry of Inorganic Ions, Russian Federation
E-mail: alenab61@mail.ru

Previously on two rat cell lines, AS-30D and PC12, we have shown that stigmatellin (an inhibitor of mitochondrial respiratory complex III) is one of the strongest protectors against Cd\(^{2+}\)-induced cytotoxicity, in addition to N-acetylcysteine and several mitochondrial permeability transition (MPT) pore inhibitors, namely bongkrekic acid (BKA) and cyclosporine A (CsA). To better understand the molecular mechanisms of the preventive action of stigmatellin, we tested its effectiveness against mitochondrial membrane permeabilization produced by such heavy metals as Cd, Hg, Cu, and Zn, as well as by Ca (in the presence of Pi) or Se (as added as Na\(_2\)SeO\(_3\)), using isolated rat liver mitochondria as a model system. The conducted experiments showed that stigmatellin exhibited the modulating effects on the mitochondrial swelling induced by these metals/metalloids in isotonic sucrose medium in the presence of Asc and TMPS (complex IV substrates) added for energization of the mitochondria in order to bypass the respiratory complexes I, II, and III inhibited by Cd\(^{2+}\), etc. In particular, stigmatellin sharply enhanced the mitochondrial swelling, evoked by selenite; however, in the same medium and under the same conditions stigmatellin as well as BKA and CsA did not produce significant effect on Cu\(^{2+}\)-induced swelling of isolated rat liver mitochondria in contrast to the high-amplitude swelling produced by Cd\(^{2+}\), Hg\(^{2+}\), Zn\(^{2+}\), or Ca\(^{2+}\) plus Pi, which significantly depressed by these inhibitors. In the light of own results and data from literature obtained during the latest time, the hypothesis suggested by us earlier (Belyaeva et al., 2004) about the possible involvement of the electron transport chain supercomplex, formed by complex I (P-site) and complex III (S-site) in the mitochondrial membrane permeabilization mediated by the MPT pore is discussed.

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9P.2 MitoTeas: Vaccinium myrtillus and Geranium robertianum decoctions improve diabetic Goto–Kakizaki rats hepatic mitochondrial oxidative phosphorylation

Fernanda M. Ferreira1, Francisco Peixoto2, Elsa Nunes3, Cristina Sena3, Raquel Seica3, Maria Sancha Santos4

1Department of Environmental Sciences (CERNAS), ESAC – Polytechnic Institute of Coimbra, Bencanta, Coimbra, Portugal
2Chemistry Department (CECAV) University of Trás-os-Montes & Alto Douro, Vila Real, Portugal
3Department of Physiology and Institute of Biomedical Research in Light and Image, Faculty of Medicine, University of Coimbra, Portugal
4Department of Zoology, Center for Neurosciences and Cell Biology of Coimbra, University of Coimbra, Portugal
E-mail: fmlferreira@gmail.com

Diet-induced metabolic syndrome, leading to obesity, insulin resistance, type 2 diabetes and related diseases are major health problems all over the world, nowadays [1,6]. A common feature to these metabolic alterations is lower mitochondrial oxidative phosphorylation (OXPHOS) enzymatic complexes activities [5]. Several chemical compounds found in plant products had proven to possess beneficial properties, being currently pointed out due to their pharmacological potential in metabolic syndrome complications [2]. In this context, we studied the effect of Vaccinium myrtillus and Geranium robertianum leaf decoctions on Goto-Kakizaki (GK) rats, a type 2 diabetes mellitus animal model. Our results show that V. myrtillus and G. robertianum leaf decoctions present significant benefits on glycaemic control and that GK rats treated during four weeks with V. myrtillus and G. robertianum decoctions presented an improvement of the evaluated mitochondrial respiratory parameters (state 3, state 4, RCR and FCCP stimulated respiration). These increased OXPHOS activities can be correlated to the high contents of quercetins found in V. myrtillus and homoeoriodictyol found in G. robertianum, that are reported to account for increased protein expression [3,4]. Therefore, these “MitoTeas” seem to be promising therapeutic agents to type 2 diabetes, regarding their high antioxidant activity coupled to their beneficial effects on glycaemic control and mitochondrial activity.

References

9P.3 A novel drug for uncomplicated malaria: Targeted high throughput screening (HTS) against the type II NADH:ubiquinone oxidoreductase (PNDH2) of Plasmodium falciparum
Nicholas Fisher1, Alasdair Hill1, Alison Mbekeani1, Alison Shone1, Gemma Nixon1, Paul Stocks1, Peter Gibbons2, Richard Amewu2, W. David Hong2, Victoria Barton2, Chandra Pidathala2, James Chadwick2, Louise Le Pensee2, Ashley Warman1, Raman Sharma2, Neil G. Berry2, Paul M. O’Neill2, Steve A. Ward1, Giancarlo A. Biagini1
1Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK
2Department of Chemistry, University of Liverpool, L69 7ZD, UK
E-mail: n.e.fisher@liverpool.ac.uk

The respiratory chain of the human malaria parasite Plasmodium falciparum lacks a canonical proton motive NADH:ubiquinone oxidoreductase (Complex I), containing instead a single-subunit, non-proton motive NDH2, similar to that found in plant mitochondria, fungi and some bacteria [1,2]. As such, the P. falciparum NDH2 (PNDH2) presents itself as an attractive anti-malarial chemothrapeutic target, and we have developed a heterologous expression system for this enzyme in the E. coli NADH dehydrogenase knockout strain ANN0222 (generously provided by Prof. Thorsten Friedrich, Freiburg) to facilitate its physicochemical and enzymological characterisation [3]. PNDH2 represents a metabolite choke point in the respiratory chain of P. falciparum mitochondria and is the focus of a drug discovery programme towards the development of a novel therapy for uncomplicated malaria. Here we describe a miniaturised assay for recombinant PNDH2 with robust assay performance measures that has been utilised for the high throughput screening (HTS) of small molecule inhibitors. The objectives of the HTS were to (i) increase the number of selective PNDH2 inhibitors and (ii) to expand the number of inhibitor chemotypes. At the time of screening, only one proof of concept molecule, 1-hydroxy-2-dodecyl-4-(1H)quinolone (HDQ), was known to have PNDH2 inhibitory activity (IC50 = 70 nM) [3,4]. This molecule was used to initiate a primary similarity-based screen of 1000 compounds from a compound collection of 750,000 compounds (curated by Biofocus-DPI). A range of chemoinformatics methods and filters were applied to the hits from this initial phase in order to perform a hit expansion screen on a further about 16,000 compounds. The chemoinformatic strategy allowed us to cover about 16% diversity whilst screening just about 2% of the compound collection. The HTS resulted in a hit rate of 0.29% and covered about 16% diversity whilst screening just about 2% of the compound collection. The HTS resulted in a hit rate of 0.29% and 150 compounds were progressed for potency against PNDH2. Of these compounds, 50 were considered active with IC50 ranging from 100 nM to 40 μM. Currently, seven distinct chemotypes are being progressed from hit to lead using traditional synthetic medicinal chemistry strategies.