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S6.P8

Sustained shivering induces adaptive mitochondrial mechanisms to buffer increased oxidative stress and maintain energy metabolism

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Eutherian mammals evolved thermogenic uncoupling protein 1 (UCP1) in brown adipose tissue mitochondria to defend body temperature in the cold by non-shivering thermogenesis replacing shivering thermogenesis [1]. UCP1 knockout (UCP1-KO) mice rely on sustained shivering in the cold but their skeletal muscle mitochondria exhibit neither dysfunction of respiration nor pronounced oxidative damage [2, 3]. Here, we studied mitochondrial reactive oxygen species (ROS) handling of muscle mitochondria from wild-type and UCP1-KO mice. We find that prolonged cold exposure increases hydrogen peroxide release rates in isolated UCP1-KO mice mitochondria, which is partially blunted by increased adenine nucleotide translocase (ANT) function. The physiological significance of deleterious ROS production during sustained shivering was further substantiated by activity of mitochondrial aconitase, a TCA cycle enzyme that is gradually inactivated by ROS. Our mouse model elucidates adaptive molecular responses to maintain mitochondrial function in muscle during sustained shivering and to buffer increased mitochondrial ROS production.

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S6.P9

Characterization of Dna2, a mitochondrial DNA maintenance protein

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Mitochondria perform energy conversion in living organisms. The function and composition of mitochondria are regulated not only by nuclear DNA, but also by mitochondrial DNA, which contains 37 genes encoding 13 proteins of the oxidative phosphorylation system, 22 tRNAs, and 2 rRNAs necessary for mitochondrial translation. Abnormal mitochondrial DNA maintenance is associated with ageing and mitochondrial diseases. We have studied the poorly understood role of DNA2, a nucleolytic protein supposed to be involved in replication and repair of mitochondrial DNA. Dna2 is a member of the helicase/nuclease family known to process intermediate 5′ flap structures occurring in DNA replication and long-patch base excision repair. We used molecular methods and conditional knockout mice to investigate the localization of Dna2 and its roles in maintaining the integrity of mitochondrial DNA in vivo. We observed that Dna2 does localize to mitochondria. Loss of Dna2 causes embryonic lethality, indicating that Dna2 is essential for mouse embryonic development.

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S6.P10

Measuring hydrogen peroxide release from isolated liver mitochondria using Amplex Red: A critical evaluation

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Fluorimetric detection of hydrogen peroxide (H^2O^2) by the horseradish peroxide (HRP)-catalysed Amplex Red assay is a well established and frequently used technique to measure mitochondrial H_2O_2 release. The technique has made significant contributions to our understanding of the fundamental mechanisms of ROS production by mitochondria and its relevance in different physiological and pathological situations. However, the reliability of the technique to detect and quantify H_2O_2 release specifically from liver mitochondria has been questioned, because there is a large unexplainable signal that occurs in the absence of respiratory substrate (no oxygen consumption), is seen in frozen-thaw samples (no membrane potential), and does not even require HRP. As a result, liver mitochondria raw values are a few times higher than in those from any other tissue and lack apparent responses to respiratory substrates and inhibitors. Mitochondrial subfractionation experiments suggested that the signal originated in the matrix. Here we show that it can be eliminated by adding PMSF to the experimental media. The dose required (100 μM) does not affect oxygen consumption, and the effect is immediate. PMSF is an inhibitor of hydrolase, whose activities were found in a number of enzymes in the P450 system in the liver. In fact, the catalytic cycle of the P450 system has a great similarity to the reactions catalysed by HRP, suggesting that the P450 system, which is highly expressed in liver, might be masking the normal H_2O_2-HRP-Amplex Red detection reaction. We have also conducted experiments without PMSF in the presence and absence of HRP and calculated the HRP dependent signal by subtraction. These two methods produced very comparable results. If calculated by either of these methods, the characteristics of H_2O_2 release from liver mitochondria are in agreement with those from different tissue sources. We suggest caution in measuring the electron transport chain dependent H2O2 release from liver mitochondria by the Amplex Red technique. Use of the raw data might result in misleading conclusions and one of the two correction methods proposed should be applied.

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