Defective mitochondrial calcium uptake and energetic mismatch in a rat model of takotsubo cardiomyopathy

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Topic(s):
Basic Science - Cardiac Diseases: Cardiomyopathies

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Background:
Takotsubo cardiomyopathy (TTC) is an acute heart failure syndrome with a typical left ventricular "ballooning" and in-hospital mortality rates of 4-5%. Human studies revealed severe energetic impairment during the acute phase of this condition.

Purpose:
The mechanisms of such energetic impairment, however, are unresolved. Here, we characterise mitochondrial function in an animal model of TTC.

Methods:
The TTC model was induced by intraperitoneal injection of isoprenaline (100 mg/kg) in 2-4 months old female Sprague Dawley rats (254 ± 25 g). Our previous studies revealed a TTC-like apical ballooning of the left ventricle at day 3 after injection, which resolved at day 7. Accordingly, cardiac myocytes and mitochondria were isolated at 3 and 7 days post-injection from TTC and control hearts (n=7, respectively). Cardiac myocytes were field stimulated and submitted to a physiological stress protocol (5 Hz stimulation plus β-adrenergic stimulation) during which the redox states of NAD(P)H and FAD (autofluorescence), cytosolic [Ca²⁺] (Indo-1) and mitochondrial membrane potential (TMRM) were determined together with sarcomere length. In a parallel set of experiments, cytosolic and mitochondrial [Ca²⁺] were determined in a fluorescence- (rhod-2/Indo-1) and patch-clamp-based approach. In isolated mitochondria, O₂ consumption (Clark electrode), reactive oxygen species (ROS) emission (Amplex red) and Ca²⁺ uptake (Ca²⁺ green assay) were assessed.

Results:
At 3 and 7 days after injection, basal respiration, Ca²⁺ uptake and ROS emission were similar in isolated mitochondria from TTC and control hearts. In isolated cardiac myocytes at day 3, systolic and diastolic cytosolic [Ca²⁺] were slightly reduced in TTC vs control hearts during maximal β-adrenergic stimulation (p<0.05), while sarcomere length and systolic shortening were unchanged. In patch-clamp experiments (with the cytosol being equilibrated by pipette solution), mitochondrial Ca²⁺ uptake was impaired despite increased cytosolic Ca²⁺ transients at day 3 in TTC cells. Accordingly, the redox states of mitochondrial NAD(P)H and FAD were moderately, but significantly oxidised during workload transitions at day 3, reflecting impaired Ca²⁺-induced stimulation of the Krebs cycle in TTC. These defects in mitochondrial Ca²⁺ uptake and redox adaptations were resolved at day 7.

Conclusions:
Defects in mitochondrial Ca²⁺ uptake provoke energy supply-and-demand mismatch which may account for energetic deficit and oxidative stress in TTC, while mitochondria appear to maintain a level of functional integrity in isolation. Further research will therefore address the detailed mechanisms of impaired mitochondrial Ca²⁺ uptake and whether therapeutic interventions to ameliorate these defects influence the phenotype of TTC.
Abstract:

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