Porphyrin-based in vivo mitochondrial respirometry.

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Introduction

A reduction in mitochondrial respiration has been associated with a variety of human disorders, like cardiovascular diseases, sepsis and septic shock. Several methods have been developed and used over the years to estimate the respirometry *in vivo*. However, most of the available methods are based on semi-quantitative measurements. Recently we introduced the Protoporphyrin IX - Triplet State Lifetime Technique (PpIX-TSLT) (Mik et al., 2006) that enables to measure the mitochondrial oxygen tension (mitoPO_2) by means of the oxygen-dependent optical properties of 5-aminolevulinic acid (ALA)-induced endogenous protoporphyrin IX (PpIX) in the mitochondria. Further development of this technique can provide a new technique to monitor the oxygenation, oxygen consumption and oxygen affinity of the mitochondrial respiratory chain *in vivo*. In this presentation we present a novel approach that allows mitochondrial respirometry by dynamic measurements of mitoPO_2 during a local blockage of microvascular flow. We show the reproducibility of the measurement and the possibility to measure in difference spices like, rat, pig and man.

Methods

The measurements were performed in rats, pigs and healthy human volunteers. The princibles of the cuteneous mitoPO2 measurements were discribed in detail elswhere (Harms et al., 2011, Harms et al., 2012). In short, ALA cream was applied at the skin to induce PpIX in the mitochondria. Photo-excitation with pulsed green light was used to induce population of the PpIX triplet-state. The lifetime of the triplet state is related to mitoPO_2 and was measured by delayed fluorescence. Oxygen consumption was determined by repeated mitoPO_2 measurements while locally blocking oxygen supply. The latter was achieved by applying local pressure with the measurement probe. The mitoPO_2 was recorded before and during a period of 60 seconds compression and the mitochondrial respirometry was analysed with the aid of the decay from the slope of the mitoPO_2/ t.

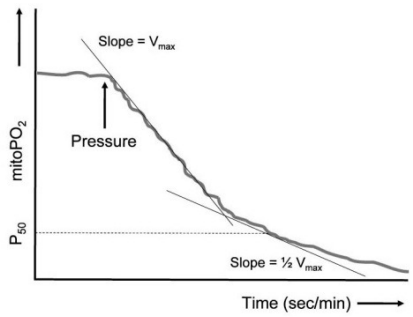
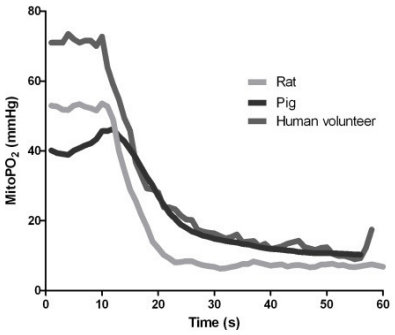


Figure 1. Principle of mitochondrial respiration by mitoPO_2 kinetics

Results

To test the inter- and intra-animal variability respirometry measurements, a total of 24 measurements were performed at for different regions on the abdominal skin of each rat. The mean PO_2 before stop-flow (P_0), the oxygen concumption (V_0) and the PO_2 at which cellular oxygen consumption is reduced to 1/2 V_{max} (P_{50}), were analysed by fitting the linear slope and were corrected for the the oxygen influx into the measurement volume. The P_0 59.4 ± 1.4 (SEM) mmHg, the mean V_0 was 4.99 ± 0.3 (SEM) mmHg/sec and the P_{50} was 3.95 ± 0.2 (SEM) mmH

The feasibility of the measurement in other spices was tested in a pilot experiment on the skin of a healthy volunteer and a pig (figure 3).



Conclusion

Our study shows the feasibility of cutaneous respirometry by a combination of PpIX-TSLT and local tissue compression. Enabling measurement of the mitochondrial parameters like, mitoPO_2, V_0 and P_{50}. We expect that clinical implementation of the method will greatly contribute to our understanding of mitochondrial function and oxygen metabolism in health and disease. For example, this technique could be useful for evaluation of dermal drugs, guiding of systemic mitochondrial therapy and monitoring of mitochondrial function in critically ill patients.

References

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