Endogenous Indicators in Mitochondria

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The mitochondrion is one of the most important cellular organelles. It provides the cell with energy, making it possible for the cell to survive and function. It can also modify the cellular signal state of cells by taking up and releasing free calcium. When the function of the mitochondria is damaged or disturbed, it can trigger the onset of cell death. Therefore, the functional state of mitochondria is a key parameter in determining cellular function. Various indicators have been exploited in order to indicate the functional state of mitochondria by measuring parameters such as the redox ability of the electron transport chain, the membrane potential, H$_2$O$_2$ production, and release of cytochrome C. However, loading indicators can cause considerable injury to fresh sample preparations because these indicators are toxic to the cell to some degree. In brain slices for example, the penetration of glucose and oxygen to deeper layers of the sample depends on simple diffusion due to the lack of a working vascular system. Cells at deeper layers can thus be damaged because of a shortage of glucose and oxygen, when perfusion is interrupted during the loading process.

Mitochondria have their own natural functional indicators—NADH (reduced form of nicotinamide-adenine dinucleotide), NADPH (reduced form of nicotinamide-adenine dinucleotide phosphate) and FAD (flavin adenine dinucleotide). NADH and NADPH are generated by glycolysis and the tricarboxylic acid cycle in the cytoplasm and have a fluorescence maximum of 450nm when excited at 360nm and 380nm respectively. FAD, however, is only generated in the tricarboxylic acid cycle and has an excitation wavelength of 450nm and a fluorescence maximum of 530nm. When the redox state of indicators is altered, excitation waves of the same wavelength do not result in fluorescence emission. As a result we can acquire endogenous optical signals that reflect the functional state of mitochondria by using Hertzian waves at appropriate wavelengths without needing to consider the loading process or the toxicity of exogenous indicators.

Although the endogenous fluorescence of the respiratory chain was discovered sixty years ago, rapid progress in the development of endogenous fluorescence imaging techniques, especially for use in brain research, has only occurred in recent years. Transcranial endogenous fluorescence imaging has been successfully used for monitoring the activity of shallow tissue in the brain under in vivo conditions, for example in investigating nervous...
excitability and neural plasticity in the sensory cortex. Endogenous fluorescence imaging has also been used to investigate pathological models such as ischemia reperfusion by conducting real-time monitoring of mitochondrial activity in the hippocampus region of brain slices. Experimental models have recently become increasingly diversified due to the use of a large number of genetically-modified animal models. Techniques for monitoring and controlling the conditions of both in vivo and in vitro experiments for optical signal detection and image processing have also been developed. Thus endogenous fluorescence imaging can now be exploited more widely and applied to a broader range of fields.

Compared with exogenous indicators, endogenous fluorescence has a significant advantage — it can be utilized conveniently in both in vivo and in vitro experiments with samples such as isolated mitochondria, dissociated cells, brain slices, and the brain. A serious and practical problem in research on central nervous system dysfunction caused by stroke and metabolic injury has been that knowledge gained from in vitro experiments is of little relevance to clinical practice. However, findings from research using mitochondrial endogenous fluorescence have shown that when conducting in vitro experiments, mitochondrial responses are similar to those in in vivo experiments. Using this technique, comparisons of in vivo and in vitro experiments have shown that ischemic mitochondrial function declines faster and the extent of the decline during the initial stages of ischemic stress is greater in in vitro experiments. During in vivo ischemic response, blood is preferentially transferred as close as possible to the site of the ischemic event. Considering that mitochondrial function is one of the first things to be affected by ischemic injury, the above results undoubtedly raise pertinent questions about the extent to which discoveries that are based on events which are downstream of ischemic mitochondrial responses in in vitro experiments can be applied. It is likely, therefore, that endogenous fluorescence will serve as a connecting point for in vivo and in vitro experiments in many fields in the future.

References