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Mitochondrial Function and Metabolic States: On the Differences between Brain In Vitro and In Vivo Conditions and Monitoring

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Abstract

Although mitochondria were discovered more than 150 years ago, monitoring of mitochondrial function in-patients in real time and in vivo is very rare. In most of the studies published in this field, isolated mitochondria or isolated cells in vitro were and are in use and therefore the technology used in those studies cannot be adapted to patient monitoring. Evaluation of mitochondrial function by optical monitoring of NADH redox state could be used in isolated mitochondria as well as in patients under various clinical situations. Therefore, we developed a medical device that could provide real time data on mitochondrial function as well as microcirculatory blood flow and oxygenation using optical based technology. In order to improve, significantly, outcome of patients in critical care medicine and other conditions it is necessary to adapt our device that was cleared by the FDA. Our preliminary results in patients indicate that our approach of monitoring of tissue vitality is the next step in improving the health of humankind.

The pioneer work of Chance, Williams, Connelly Theorell and other collaborators, in the early 1950's, opened up new possibilities enabling the study of mitochondrial function in vitro (1-7), and later on in vivo monitoring became a reality. The shift from studying mitochondrial NADH redox state in isolated mitochondria to higher cellular and tissue organization level started in the late 1950's (8-11). The main breakthrough occurred in 1962, a year that the group of Britton Chance published at least 6 papers describing the in vivo monitoring of NADH fluorescence in various organs in anesthetized rats (12-15).

Figure 1 presents the main elements of a typical brain tissue, namely, a neuron and a glial cell as well as synapses and 2 capillaries. Most of the ATP needed for the cellular activities is produce by the mitochondrion. There is a direct coupling between the rate of ATP production and activity of various pumps, such as Na-K-ATPase, and the supply of oxygen by the capillaries. This coupling is missing when isolated mitochondrial function is evaluated under in vitro conditions. It is possible to monitor various parameters, of the brain in vivo that represents the various activities related to oxygen supply, demand and the balance of energy in the tissue as well as integrated brain functions. The details of the multiparametric monitoring system developed in our laboratory are presented in a recently published book (16).

Mitochondrial function and its metabolic states under in vivo condition are completely different from the definition made for isolated mitochondria by Chance and Williams in 1955 (5) as seen in the left side of the Figure 2. In order to compare the in vitro to the in vivo states, only four states (2 -5) are presented. The "resting" state of the mitochondria in vitro was defined as "State 4", where NADH was 99% in the reduced form, and ADP was the rate limiting substance. If ADP is added to a suspension of mitochondria, ATP synthesis will be stimulated, oxygen consumption will increase and the rate limit will be determined by the activity of the respiratory chain. During this State 3, or the

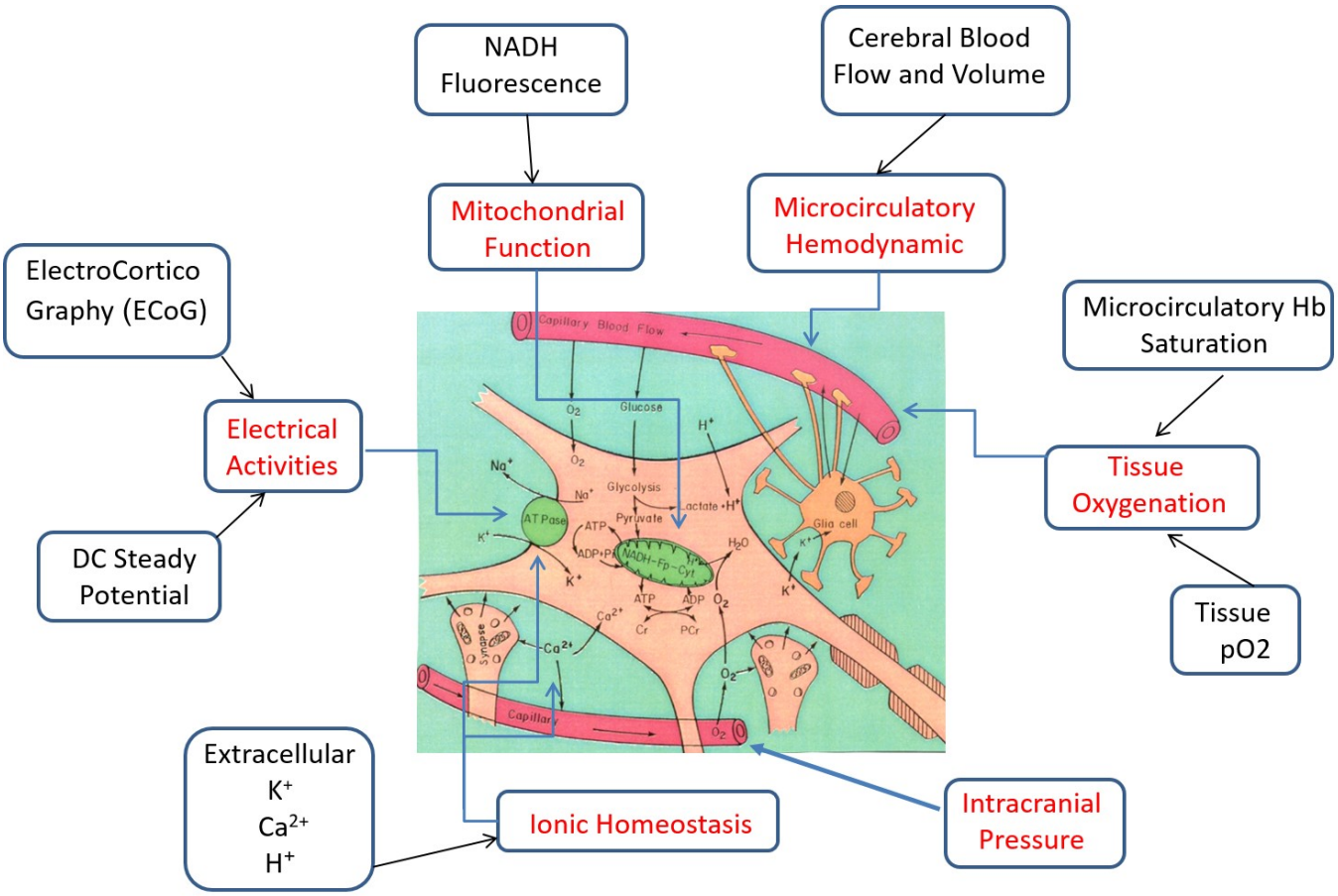

FIGURE 1: Schematic presentation of the main structural components of a cerebral cortex tissue that could be monitored in vivo using various technologies.

"active state", the NADH redox state will decrease or become more oxidized (about 50%). When the "resting" mitochondria are deprived of oxygen, the activity of the respiratory chain will stop and NADH will reach its maximum redox state (State 5).

A definitive description of the mitochondrial metabolic state has never been given for in vivo conditions. Therefore, we described the in vivo mitochondria conditions as recorded by NADH fluorescence in a representative tissue or organ – e.g. the brain. While the range between minimal NADH (~0) and its maximal level was determined in vitro, it is almost impossible to determine it in the intact brain or other organs in vivo. For example, State 2, with a substrate free medium, could not be achieved in vivo since the tissue would die due to the lack of substrate. On the other hand, the maximal level of NADH (State 5) could be monitored in vivo under complete deprivation of oxygen by anoxia or complete ischemia. We used the changes in NADH levels monitored in vivo to create a new scale ranging from a maximal definite point to the minimal level as shown in the right side of figure 2. Details of this approach have been published (17).

As seen in Figure 2, the maximal NADH level is achieved under complete O_2 deprivation that can be induced both under in vitro and in vivo conditions. This signifies that this definitive point can be used to determine State 5 in vivo as well. The problem is to determine the metabolic state of a tissue in an in vivo situation. If we adopt the in vitro value of a resting state (State 4), this would signify that the increase in NADH during state 5, induced by anoxia (0% O_2), would be only 1%. According to all in vivo studies, this is not the case, and during anoxia the increase in NADH is larger than the decrease under state 4 to 3 transitions. Figure 2 (right hand panel) illustrates that the observed level of NADH increase is indeed larger than the decrease. Therefore, we concluded that, under in vivo conditions, the "resting" metabolic state of the brain is found between States 4 and 3

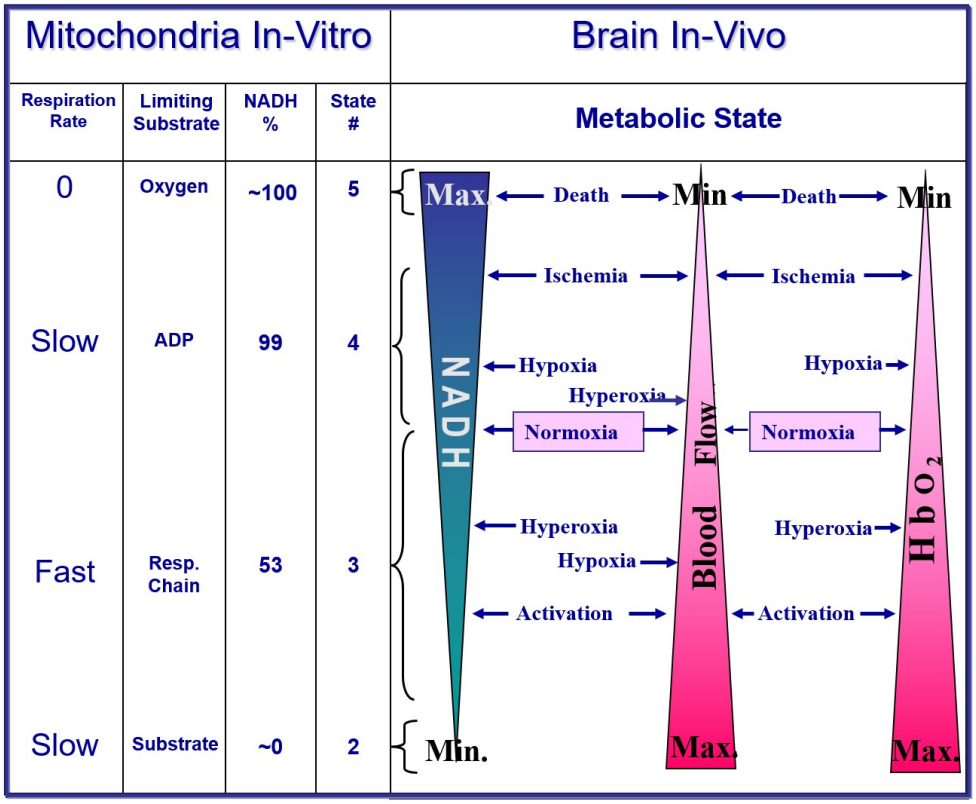

FIGURE 2: Comparison between mitochondrial metabolic states in vitro (left side) and the brain in vivo (right side).

rather than in State 4 as defined in vitro (17). In order to determine the maximal levels of NADH in vivo it is almost impossible to use cyanide, therefore, we used 100% nitrogen until the cessation of respiration and the recovery to air was monitored. It is almost impossible to achieve the minimal level of NADH (maximum oxidation) in vivo as compared to the level determined in state 2 when substrate availability is minimal. We were able to inject a non-fluorescing uncoupler (Penta Chloro Phenol) into the brain and NADH became more oxidized (18). Using fiber optic fluorometry, we measured the brain in anesthetized as well as awake rats exposed to many perturbations in vivo and measured the changes in NADH redox state together with other physiological parameter shown also in Figure 2 (17, 19).

The main differences between the in vitro and in vivo situations are as follows:

1. The isolated mitochondria are not inter connected to other intracellular organelles and components.

2. The amount of oxygen available in the mitochondrial medium is not regulated by changes in blood flow or hemoglobin oxygenation under in vitro conditions.

3. One of the largest oxygen or energy consumers i.e. various ions pumps, are missing from the isolated mitochondria preparation.

4. When ADP is added to State 4 mitochondria, ATP will be synthesized but will not be consumed in parallel as occur under in vivo conditions.

5. The isolated mitochondria preparation are not exposed to systemic hemodynamic and other physiological parameters changes occurred in the organism.

Since 1962 a very large number of papers described the use of NADH fluorescence monitoring in intact animals exposed to variety of physiological and pathological conditions. Mitochondrial NADH was monitored in various animal models exposing most of the organs in the body to various perturbations.

In the right side of Figure 2, the relationship between brain NADH redox state and microcirculatory blood flow (CBF) and oxygenation (HbO_2) are presented for various perturbations. The correlation between the responses of the three monitored parameters to various perturbations are not

identical. For example, under ischemia, the CBF and HbO_2 decreased and NADH in the brain is elevated. In hypoxia, CBF is elevated in order to provide more oxygen due to the decrease in the oxygenation of the blood but NADH was elevated. In hyperoxia, induced by hyperbaric oxygenation, the relationship between the three parameters are different. Although blood flow decreased, due to vasoconstriction, the NADH became more oxidized due to the increase oxygen supply by the higher level of oxygen in the plasma of the blood and the elevated HbO_2 saturation in the microcirculation. The main problem is how to create a more oxidized NADH in vivo. In the brain we used various models of brain activation starting with hyperoxia followed by epileptic activity and cortical spreading depression and the results were discussed recently (16). The involvement of the micro-vascular compensation for extra oxygen needed under brain activation could be measured only under in vivo situation and not in vitro in isolated mitochondria.

The crucial questions that remain open are:

1. Could we interpret the in vivo data according to the concept developed for in vitro conditions?

2. Can we compare and define the resting state (4) and active state (3) under in vivo situation to the in vitro definitions?

3. How could we overcome the issue of the missing technique of calibration the NADH signal monitoring in vivo in absolute values?

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