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Non-invasive approaches to tissue bioenergetics

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The great upsurge of interest and activity in non-invasive methods that will permit the qualitative and quantitative studies of tissue bioenergetics is consonant with the change of health-care attitudes from invasive to non-invasive procedures.

This discussion embraces several methods for studying different kinds of bioenergetic deficiencies of a variety of tissues, and a special emphasis is placed upon brain and hypoxic/ischaemic tissue damage, presented here in the context of new instrumental approaches.

^{31}P-n.m.r.

^{31}P-n.m.r. now can be recognized as a 'gold standard' of tissue hypoxia and quantification of the metabolic load placed by tissue fraction upon the capacity of the tissue for oxidative phosphorylation. The application of the simple Michaelis–Menten algorithm, $V/V_{max.} = (1/1 + K_m)/[ADP]$, to skeletal muscle is simple and straightforward: V can be varied by exercise stress and the plateau of the hyperbolic relationship between work and P_i/phosphocreatine (PCr) can be readily quantified from values at low $V/V_{max.}$ where acidosis, etc. do not interfere [1]. This algorithm has been applied as well to liver in the natural animal, and in the transgenic liver in which creatine kinase is expressed at significant levels [2]. Using various metabolic loads (ammonia, ornithine, etc.), it has been possible to demonstrate Michaelis–Menten regulatory functions of ADP and to quantify the $V_{max.}$ as well. The application of these principles to the brain is more difficult and we are only beginning to learn the physiological response of the brain to functional activity. There are a number of problems. First, functional activity of the brain appears to be highly localized, as demonstrated by the results of positron emission tomography (p.e.t.) and magnetic resonance imaging (m.r.i.). Secondly, vasomotor responses to functional activity appear to be large and rapid, as determined especially by m.r.i. and, most recently, by optical methods (described below). Thus, hyperaemia and hyperoxygenation appear to accompany localized metabolic activity of the brain, generating ideal conditions for control of metabolism exclusively by ADP. N.m.r. testifies that the ADP level for the global average of metabolic activity appears to be close to the mid-range for ADP control (i.e. PCr/P_i of ~3 for the adult brain, or $V/V_{max.}$ ~50%). In the neonate brain, $V/V_{max.}$ may rise to higher values (~70% for pre-term neonates) [3,4]. However, perturbation of brain oxidative metabolism by varying metabolic work is not currently feasible, even through the use of electroshock in therapeutic studies (L. Gyulai, personal communication). Thus we know the set point for brain oxidative metabolism to be > 50% of $V_{max.}$ from n.m.r. studies, but we are not able to quantify $V_{max.}$ under normal conditions, or in conditions where brain oxidative metabolism is impaired by the variety of diseases discussed in this symposium that may impair mitochondrial function *in vivo*.

Abbreviations used: DO_2, O_2 delivery; EDH, epidural haematoma; m.r.i., magnetic resonance imaging; p.e.t., positron emission; SDH, subdural haematoma; VO_2, O_2 uptake.

Optical studies

N.m.r. and e.p.r. measure tissue oxygen with significant difficulties, and direct optical measurement of haemoglobin in the arteriolar, capillary and venolar beds has been under development for some years for brain studies [6]. While much of the early work has been focused upon attempts to measure cytochrome oxidase, newer knowledge on the effects of light scattering upon *in vivo* quantification of optical signals suggests that current methodology for cytochrome oxidase measurement is inadequate and that severe cross-talk between cytochrome oxidase and haemoglobin signals has, in a number of laboratories, obfuscated unique measurement of cytochrome oxidase over the range of physiological and pathological conditions. Another problem of cytochrome oxidase studies relates to mitochondrial function. The cytochrome electron-transfer components, particularly cytochrome oxidase, have redox states which vary with the local tissue O_2 concentration, even though the rate of oxidative phosphorylation may be maintained over those ranges. On the other hand, NADH itself, which generally is inaccessible by non-invasive methods, has a steady state related to ATP synthesis. Thus, in contrast to n.m.r., where the requirement for ATP synthesis impacts directly upon the P_i/PCr ratio, cytochrome oxidase may change its steady state over very wide ranges without becoming rate-limiting due to its extraordinary reactivity towards O_2; there is little effect upon the rate of ATP synthesis.

Haemoglobin, on the other hand, presents a very sound optical signal from the tissue surface and from well within the tissue, particularly as the technology is based upon photon-migration theories applied to tissue observations deep within functioning tissues.

Apparatus designed to measure changes of haemoglobin oxygenation as a function of physiological distress are based upon the principles of G. A. Millikan's 'oximeter' as applied initially to cat soleus muscle and, during World War II, to the human earlobe [7,8]. The recognition that red light gave better signals than the green lights used by Millikan was first tested in detail by Matthes and Gross [9], who showed light transmission through the human hand using deep-red light. The tissue oximeter has exploited these wavelengths for finger pulse oximetry, and Jobsis has devised multiwavelength devices for measuring cytochrome oxidase and haemoglobin in the neonate brain by what he termed 'transillumination' [10].

The realization that no photons enjoy an unperturbed trip through living tissue at distances in excess of a millimetre [11,12] has led to a much better understanding of the limitations of tissue haemoglobinometry and to the development of instruments which, on the one hand, provide only qualitative information (continuous light devices) [13] and, on the other hand, realize the possibilities for quantification, namely, time- and frequency-domain techniques [11]. Thus two distinct categories of instruments have emerged: those that measure trends in concentration of oxy- and deoxyhemoglobin and quantify only the changes of concentration using an assumed pathlength or pathlength factor; and instruments that measure continuously the pathlength or absorbance and are capable of measuring not only changes of concentration but absolute concentrations, from which desaturation of haemoglobin during hypoxia/ischaemia can be quantified. Often, there is confusion between those two kinds of instruments, particularly in the minds of manufacturers, as noted in this meeting where quantification of haemoglobin saturation in brain may be claimed for instruments which are capable of measuring concentration changes only, termed trend indicators.

Continuous-light trend indicators

Much can be learned about the brain using extremely simple indicators of oxygenation trends that is useful to both basic science and clinical studies.

Instrumentation

Following the ideas of Glenn Millikan [7,8] and transposing them from green and red wavelengths to the absorption peaks of deoxy and oxyhaemoglobin at 760 and 850 nm respectively, a dual-wavelength spectrophotometer can be constructed. This spectrophotometer is sensitive to appropriate portions of the difference of absorbance at the two wavelengths, and measures oxygenation changes and not to blood concentration changes, or by recording appropriate portions of the sum of the responses of the two wavelengths to respond exclusively to blood concentration changes but not blood oxygenation changes, over the range of hematocrits expected under physiological conditions. Applications, to name but a few, include hypoxia, ischaemia, brain bleeding, detection of bioenergetic insufficiency and cognition.

Brain hypoxia in cardiac arrest

Figure 1 illustrates the response of the human frontal region to cardiac arrest as observed clinically under conditions of evaluating the response of

cardiac defibrillators [14]. The figure illustrates, as a function of time, the following parameters recorded clinically: from top to bottom, the cessation of activity in the integrated and analogue EEG, the earlier fall of MABP from normal to that of the fibrillating heart, and the very rapid deoxygenation response of the frontal cortex, with a half-life of ~20 s and plateauing out near what would be expected to be 100% deoxygenation; and the skeletal muscle deoxygenation measured with a separate unit, which shows a delayed deoxygenation and a delayed recovery with respect to the brain oxygenation. On restarting the heart activity with the defibrillator, we observe a very rapid hyperaemic response of the brain, together with resumption of electrical activity, and a delayed response in the peripheral circulation of the muscle. The hyperaemia lasts 2–3 min before restoration of the initial resting oxygenation of the brain. The chart indicates clearly the possibility of hyperoxygenation of the brain in reflow after low-flow hypoxia/ischaemia.

Another application of trend indicators to brain hypoxia is evaluation in the decerebrate subject of the change of oxygenation upon turning off the respirator in intubated subjects; if no change is observed, the brain can be considered to have been experiencing chronic hypoxia.

Many other applications are available, but generally the limitations of these devices are the inability to quantify saturation, and the inability to sharply localize the region of the brain from which the signals are originating.

Brain bleeds

Asymmetries of brain blood absorbance observed during even minor haemorrhage have been studied in detail by C. Robertson and colleagues [15] at Baylor. Calibration of the absorbance signals on the contralateral hemisphere, and transferring the probe to the ipsilateral hemisphere where injuries have occurred, clearly indicate epidural, subdural and cranial bleeds. The method is much more rapid and accessible to the injured patient than in the 'gold standard', CT.

Figure 2 illustrates most vividly the asymmetric signal in the case of subdural haematoma (SDH) and epidural haematoma (EDH) to be ~0.8 in O.D. difference whilst the intracranial bleeds give a signal of 0.3 in O.D. difference [15]. The controls, post-operative signals, give values of <0.05 O.D., giving a signal-to-background ratio of 10–20.

Detection of bioenergetic insufficiency

In this case, most of the subjects studied exhibit peripheral weakness, lactic acidosis, early fatigue,

Figure 1

Optical measurement of brain and leg hypoxia initiated by cardiac arrest in a human subject on which a defibrillator was tested

Additional traces indicate the electroencephalogram (EEG) power and amplitude and their diminution, together with the mean arterial blood pressure fall (1 mm Hg ≈ 133.3 Pa). A distinct hyperaemia is observed on restarting the heart with the defibrillator, concomitant with restarting of the heart beat.

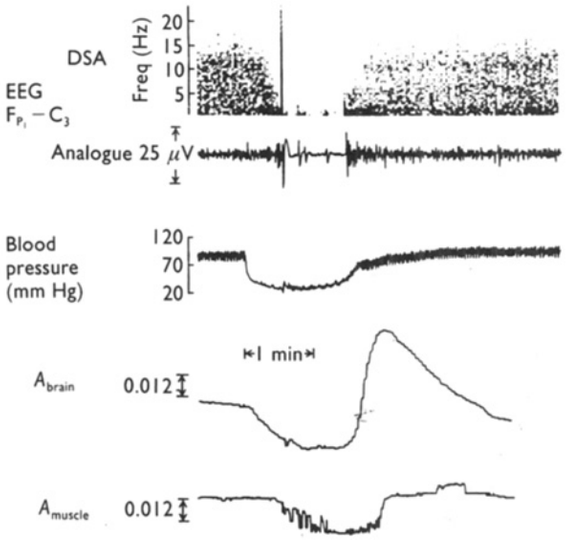
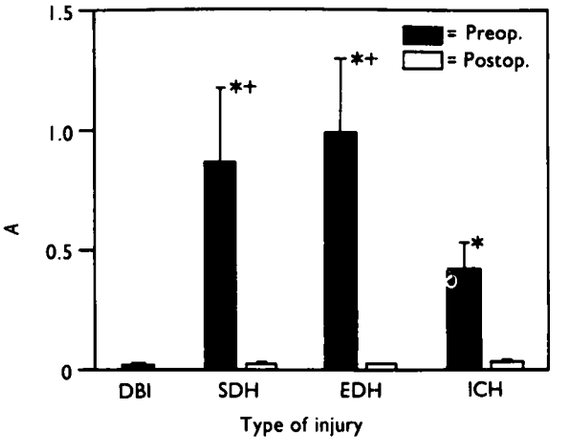


Figure 2

Sensitivity and specificity of the optical method for detecting brain bleeds due to heat injury (DBI), epidural (EDH), subdural (SDH) and intracranial (ICH)

The asymmetric absorbancies on the optically determined on the head, are shown for subdural, epidural and intracranial bleeds.



and indeed myoglobinuria, and bioenergetic capability of the limbs is of interest. Thus the probe is applied, for example, to the gastrocnemius muscle, and walking exercise provides the desirable indications of trends of oxygenation induced by exercise. In the normal individual, the uptake of oxygen (VO_2) by the mitochondria exceeds the delivery of oxygen (DO_2), and deoxygenation occurs during exercise. In a number of cases, where biopsy has verified the presence of mitochondrial disease, the opposite appears to be true; VO_2 is so small relative to DO_2 that a significant hyperoxia occurs during exercise. These converse responses have enabled the distinction of metabolic disease in McArdle's phosphofructose kinase and cytochrome oxidase deficiencies, all of which show hyperoxygenation in exercise, whereas all normals, together with a particular patient having carnitine phosphoryl transferase deficiency (which did not affect the bioenergetic capability), show deoxygenation during exercise.

Cognition

The great interest shown in establishing functional activity of the brain by m.r.i., p.e.t., SPECT and MEG has highlighted technical problems which can only be approached with significant patient confinement, isotopic injection and, in all cases, with a very large capital investment in medical equipment. The possibility that the haemoglobin trend indicator would reveal changes of tissue oxygenation accompanying electrical activity has been explored initially in our laboratory [16] and more recently by Tamura [17] and Gratton [18]. Generally, responses of the occipital cortex are well localized and relatively large in signal change. By applying an optical detector to the occipital cortex, we have been able to show on/off responses on the adult human brain in the occipital region which afford identification of the nature of the change, found to be difficult using m.r.i. [19].

In the case of Figure 2, a more sophisticated optical device was used in which the pathlength changes were measured and an increase of optical pathlength corresponds to a decrease of light absorption at 754 nm, corresponding to oxygenation during stimulation, and conversely deoxygenation during the transition from activity to rest. The histogram displays the results of trials on a particular individual and verifies that the mean change of pathlengths is ~2 cm in a mean pathlength of ~20 cm.

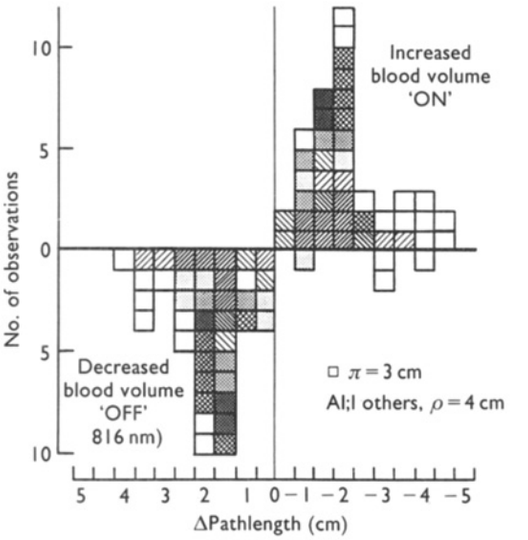
Other responses have been observed, for example, in the parietal region due to sensory motor stimulation, and in the frontal region due to cognitive responses, for example, abstract thinking. Increased blood concentration changes, and, where measured, oxygenation increases as well, identify the brain as being quite different from muscle. In the brain, an increase of VO_2 causes a luxury perfusion or excess response so that the brain becomes more oxygenated. The portion of the brain in functional activity is favoured in tissue oxygenation over that of surrounding resting areas.

In the case of the stimulation of abstract thinking in the frontal region, Fourier transformation of the signal reveals characteristic low-frequency repetitive responses that may be part of a scanning procedure involving multiple centres involved in mentation [16].

Figure 3

Blood volume increases in the occipital region initiated by flashing a checkerboard pattern before the eyes of a human subject

All trials are made on the same human subject; each box represents a single study of 'on' and 'off' responses. A shortening of the pathlength is expected and indicates a greater haemoglobin content of the brain tissue during the visualization of the checkerboard pattern in the occipital region. The observed quantity of change in optical pathlength observed with a phase-modulation spectrophotometer. The shortening of optical pathlength is related to a increase of absorbance at the measuring wavelength (816 nm). ρ is the separation of input/output fibres.



Summary

It is clear that continuous light affords a very limited window of opportunity for quantitative spectrophotometry of brain tissue. However, the number of qualitative applications which are available exem-

plify how this extremely simple technique can be applied to important medical problems.

The development of devices which can measure directly the oxygen saturation of the brain is more complicated yet affords the essential data necessary for clinical decisions on the degree of hypoxia which may be critical for neuronal survival. We can predict that such devices will be available for reliable operation shortly and will complement the existing continous-light devices.

The information from time- and frequency-domain equipment can be employed in two ways, either directly to give saturation of haemoglobin by the dual-wavelength algorithms or to provide path-length information continuously to the continuous-light devices, as many of these are commercially available and afford realistically only trend information. Thus, quantitative brain oximetry can be obtained from continuous light devices with the input of pathlength information from time- and frequency-domain systems.

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