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New Techniques, New Opportunities, Old Problems

THE STUDIES OF Wyrwicz *et al.*[1,2] on the elimination of fluorinated anesthetics from rabbit brain *in situ* using magnetic resonance spectroscopy (MRS) were among the first applications of this tool to problems of direct interest to anesthesiologists. The results of these studies, both surprising and controversial, suggested that anesthetic elimination from brain was prolonged well beyond that time predicted from brain blood flow and brain anesthetic solubility.[3] The articles in this issue of ANESTHESIOLOGY by Litt *et al.*[4] and Mills *et al.*[5] use this same new technology to elucidate and further define the issues raised by Wyrwicz. A discussion of the differences between the findings of Wyrwicz and collaborators and the current investigators can be found in their respective papers and in a paper by Strum *et al.*[6] Because an appreciation of these differences requires some understanding of the basic principles and techniques of MRS, in this editorial, we discuss the origin of the magnetic resonance signal and some problems associated with its interpretation. Additionally, we briefly review the growing role, both in the present as well as that foreseen to be in the future, of MRS in the investigation of problems related to anesthesia and intensive care.

Magnetic Resonance Spectroscopy

The radio frequency (rf) signals emitted when paramagnetic nuclei are placed in a strong, uniform magnetic field and then stimulated at their resonant frequencies by rf pulses produce the basic information from which magnetic resonance spectra are derived.[7-9] The specific rf spectrum (frequency and amplitude) obtained depends on the nuclei involved (^{31}Phosphorus, ^{1}Hydrogen, ^{13}Carbon, ^{19}Fluorine, ^{23}Sodium) and the chemical environment of the particular nuclei. The signal is mathematically transformed (fourier) to delineate frequency relationships, and the integrated intensity of the fourier transformed signal is proportional to the concentration of each nuclear species present. Thus MRS provides biochemical information related to identity as well as quantity. The technique is non-invasive. No tissue damage has been detected with current magnetic field strengths and rf energy deposition.[10] In addition, the technique is primarily sensitive to unbound, metabolically active compounds, and does not introduce artifactual losses of labile compounds such as are seen with various techniques that combine biopsy and rapid reduction of tissue temperature (freeze clamping). Thus, measurements can be made during control, insult, and recovery in the same animal; the technique is compatible with studies of survival and outcome; and repeat studies can be made on both human volunteers and patients without the risks associated with x-ray radiation or radioisotopes.[10] In addition, studies of high energy phosphorus compounds can finally be made in humans; studies that, in the past, have been done only rarely because of the need to biopsy the tissue to be studied. Considerable work has examined changes in phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphorus (P_i), and intracellular *p*H (pH_i) using ^{31}P MRS; and lactate and selected amino acids using ^{1}H MRS (see below). Advances in MRS technology now allow for measurement of multiple nuclei, such as ^{1}H and ^{31}P, nearly simultaneously expanding the amount of information that can be obtained.[11] In addition to providing information on the intracellular concentrations of various compounds, MRS can provide information on the total metabolic[12] flux or the flux between two compounds.[13,14] Thus, non-invasive studies of cellular kinetics are possible.

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Address reprint requests to Dr. Smith: Department of Anesthesia, 7 Dulles Building, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104.

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Limitations of MRS

MRS operates at the limits of sensitivity set by the physical limitations of rf transmission and detection systems. Under optimal conditions, a single rf pulse can detect, with an accuracy of 10%, the PCr peak in adult human muscle. However, many factors impair this performance to the point where averaging times of several minutes may be required to obtain the above-mentioned accuracy. The signal-to-noise ratio of the rf signals is on the order to 4–10:1, depending on coil design and the organ system to be studied. The signals are easily obliterated by other rf signal sources, or inhomogeneities introduced into the magnetic field by the subject under study. The high noise level requires relatively long signal acquisition times, making MRS unsuitable for studying certain rapidly changing events (*i.e.*, <1 s).

Problems with Signal Localization

The majority of MRS studies to date have used surface coil techniques (a combined rf receiver and transmitter coil directly over the organ of interest) with 90° rf pulses. These surface coils "see" a volume of tissue directly under the coil and extending in depth roughly proportional to the radius of the coil.[7,9,15] In most animal studies, interfering tissues must be removed, such as the overlying muscle for studies of brain. In some situations, the interfering tissue is not easily removed; for example, fatty portions of the head in studies of lactate accumulation or anesthetic elimination. However, depth resolution can be obtained by various pulse techniques that may help diminish the intensity of the interfering signal or provide better localization.[16–18] More recently, magnetic gradient techniques, "rotating frame methods," similar to those used for proton MRI, have been applied to phosphorus and fluorine.[19,20] The size and shape of the surface coil may also be critical in decreasing the contribution of interfering signals. Finally, animals with widespread, relatively uniform insults (such as global hypoxia) may be easier to study than those with localized heterogeneous insults (carotid artery ligations, hypotension, middle cerebral artery ligation). This issue of tissue localization may have direct bearing on the differences in the findings of Wyrwicz *et al.*[1,2] compared to those of Litt *et al.*[4] and Mills *et al.*[5] In the latter papers, careful attempts to select brain tissue and avoiding surrounding fatty tissues produced inhalational agent elimination curves similar to the curves obtained with classical approaches.

Studies in Animals

Most animal studies have focused on changes in brain high-energy metabolites as a function of type of insult, duration, and return of these metabolites toward normal concentration after restoration of oxygen.[21–26] Below a Pa_{O_2} of 20–25 mmHg, there is an inverse relationship between PCr/Pi (which can be determined by MRS) and tissue oxygen concentration. These changes seen with hypoxic-hypoxia can be distinguished from changes that occur with ischemic insults.[24–31] The ability to measure changes in *p*Hi by quantitating the changing chemical shift of Pi allows non-invasive measurement of this variable,[32] and *p*Hi determined by MRS agrees with values obtained by other techniques.[33]

Studies of heart using MRS are more difficult than brain, because signal acquisition must be coordinated to the movements of the heart and chest. Ligeti *et al.*[34] studied cardiac metabolism in cats and dogs, and showed that, in the cat, changes in Pi/PCr correlated to changes in work (pacing), while, in the dog, there was a much smaller decrease in the levels of high energy phosphate compounds or increase in Pi, suggesting species differences in the precision of regulation of mitochondrial energy production. In a series of three studies,[35]*† MRS and MRS combined with NADH fluorescence were used to demonstrate that NADH levels, as well as changes in ADP concentration, may be important in cardiac energy regulation. In this issue of ANESTHESIOLOGY, McAuliffe and Hickey[36] have shown, using ^{31}P MRS, that the decreased contractility produced by halothane in the neonatal heart occurs without changes in high energy phosphorus metabolites or *p*Hi.

^{13}C has a great potential for MRS studies, but its low natural abundance, low signal strength, and high cost have slowed progress in this area. Sherry *et al.*[37] recently used ^{13}C MRS to study substrate preference in the perfused guinea pig heart, and verified that lactate is used preferentially to glucose. In addition to the use of ^{19}F to study anesthetic uptake and elimination,[1,2,4,5] Eleff *et al.*‡ reported the initial attempts to measure cerebral blood flow using the washout of freon 23 (CHF_3).

Studies in Humans

Until recently, MRS studies in humans have been limited by the bore size of available magnets. At present,

* Katz A, Koretsky AP, Balaban RS: The effects of increased work on high energy phosphates and NADH levels in the perfused heart: A 31P NMR and fluorescence study. Society of Magnetic Resonance in Medicine, 5th Annual Meeting, Montreal, August, 1986.

† Koretsky AP, Balaban RS: Respiratory control by mitochondrial NADH and extra-mitochondrial phosphates: A 31P NMR and fluorescence study. Society of Magnetic Resonance in Medicine, 5th Annual Meeting, Montreal, August, 1986.

‡ Eleff S, Ligetti L, Osbakken M, Subramanian H, Chance B, Leigh JS: Brain blood flow using 19-F NMR and freon 23 washout with simultaneous phosphorus metabolite monitoring. Society of Magnetic Resonance in Medicine, Fifth Annual Meeting, Montreal, August, 1986.

most studies have been done on skeletal muscle[38] or on the neonatal brain.[39-42]

MRS has been used for both diagnosis and treatment of muscle disorders. In a patient with a mitochondrial myopathy due to lack of cytochromes b and c, ^{31}P MRS was used to monitor the results of a therapy based on vitamin K_3 and C administration,[43] and ^{31}P MRS has proved essential in noninvasive followup.[44] In addition, MRS has been used for studies of muscle exercise performance, for the development of concepts concerning muscle energy utilization and work, and in the study of genetic metabolic diseases.[12,45]

With respect to brain, high field magnets (1.5 T) situated within neonatal intensive care units have permitted repeated measurements on severely compromised infants (nearly 500 studies).[41,42] ^{31}P MRS spectra from adult human brain have also been published.[46-48] MRS of the adult brain requires localization techniques to avoid muscle contamination, and these studies give values of PCr/P_i similar to the adult dog brain.[23] Most recently, Radda *et al.*§ have demonstrated the ability to obtain spectra from a 30 ml tissue volume of human brain in less than 10 min, with a coefficient of variation of 10–20%. This improved selectivity further increases the potential clinical utility of ^{31}P MRS in brain.

Summary and Future Opportunities

The major strength of MRS is that it makes possible analytical biochemistry in intact body tissues. Because the technique is noninvasive, organs of interest can be measured repeatedly. Furthermore, two key parameters of energy metabolism are quantified; namely, phosphate potential and velocity of oxidative metabolism. Evaluations of metabolic heterogeneity seem possible.¶ Though most studies to date have been done on anesthetized animals, studies in sedated or restrained animals are possible. The period of observation can extend from hours to months, and, with advances in implanted coils and depth measurement, the range of organs that can be studied is increasing. The recent success with MRS measurements using larger bore magnets suggests the possibility of clinical utility. As with other new techniques, measurements with MRS may provide data at variance with earlier studies; for example, values of Pi and ADP obtained with MRS are much lower than values determined by enzymatic analysis of freeze or microwave clamped tissue extracts. Explanations of these differences may, in some cases, bring to light new concepts and hypotheses. In others, it may only highlight the difficulties in applying such a new technology. At present, many of the studies using MRS have confirmed early and extended findings using other techniques. Now, however, MRS is beginning to provide answers to questions about physiology and biochemistry best answered using noninvasive approaches.

DAVID S. SMITH, M.D., PH.D.
Assistant Professor
Department of Anesthesia

BRITTON CHANCE, PH.D.
Emeritus University Professor

University of Pennsylvania
Philadelphia, Pennsylvania 19104

§ Radda GK, Oberhaensli RD, Hilton-Jones D, Blackledge M, Bore PJ, Rajagopalan B, Styles P: Energetics of the adult human brain studied by ^{31}P NMR. Society of Magnetic Resonance in Medicine, Fifth Annual Meeting, Montreal, 1986.

¶ Chance B, Nioka S, Smith DS, Leigh JS Jr: Biochemical heterogeneity in brain ischemia. Society of Magnetic Resonance in Medicine, Fifth Annual Meeting, Montreal, August, 1986.

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