Nuclear magnetic resonance—a ‘magnetic eye’ on metabolism

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Introduction

Nuclear magnetic resonance (n.m.r.) spectrometers have been a common sight in chemistry laboratories for at least 20 years, but only recently has this technique become sufficiently sensitive for studies to be made on metabolites in living tissue. The result has been an explosion of interest in the last 5 years and it is now conceivable that this non-invasive technique could enable clinicians to study metabolite concentrations and intracellular pH in human subjects. The present state of the art falls a long way short of this, however, and in this review we will describe the short history of the technique and attempt to predict future developments. Inevitably we will concentrate on our own field of interest, n.m.r. studies of perfused organs or tissues. The reader who is particularly interested in spin-imaging (or zeugmatography) in which the magnetic relaxation properties of tissue water are used to obtain a two-dimensional image, is referred to the papers presented at a meeting of The Royal Society (Williams, Andrew & Radda, 1980).

Underlying principles and techniques

Most of this review can be understood without any deep appreciation of the fundamentals of n.m.r. (for an introduction see Knowles, Marsh & Rattle, 1976). Like most spectroscopic techniques it involves the perturbation of molecules by an external energy source and the measurement of a resulting signal. N.m.r. is unusual, however, in that the signal is produced by perturbing the nuclei rather than the electron shells of atoms, and some of its properties and techniques can only be fully comprehended from the standpoint of quantum mechanics.

The basic property exploited in n.m.r. is the ‘spin’ of certain atomic nuclei [e.g. the hydrogen atom or proton (1H), 31phosphorus (31P), and 13carbon (13C)]. Such nuclei, when placed in a magnetic field and irradiated with radiofrequency electromagnetic radiation, will absorb energy. There are enormous differences in the frequencies at which different nuclei (e.g. 1H, 31P, 13C) absorb energy in a given magnetic field and much smaller, but easily distinguishable differences caused by the local environment of the nuclei. These small differences result in a spectrum of peaks which characterize the local environment of the atoms in a compound. Most existing n.m.r. spectrometers were originally intended to enable organic chemists to study the structure of fairly simple compounds in this way; they include design features which make the study of biological samples unnecessarily difficult.

N.m.r. is much less sensitive than other forms of spectroscopy, although the sensitivity varies from one atomic nucleus to another. Of the three considered here the proton gives the strongest signal but is so abundant in living tissue that special techniques are necessary to resolve individual signals from the multitude of overlapping peaks. To date, as far as living systems are concerned, proton n.m.r. has only been successfully applied to erythrocytes by the use of special pulse techniques.

The naturally occurring isotope of carbon is 12C, which has no nuclear spin. Only 1% of natural carbon is 13C, which has spin but gives a much weaker signal than the proton. Its usefulness comes from administration of 13C-labelled compounds whose metabolism can then be followed continuously. 13C has one other advantage in that the resonant frequency range is very large. This means that even very closely related molecules and functional groups can be distinguished, e.g., the α and β anomers of D-glucose (Cohen, Shulman & McLaughlin, 1979).
Most n.m.r. work on intact biological systems has concentrated on $^{31}\text{P}$, the naturally occurring isotope of phosphorus. This nucleus gives signals one-sixth the strength of the proton, but there are several important phosphorus-containing metabolites present in the cell at a sufficient concentration to be measured, e.g. ATP, ADP and inorganic phosphate. An added advantage is that the frequency of the inorganic phosphate resonance is an indicator of intracellular pH (see also below).

In order to overcome the low sensitivity of the technique, it is almost always necessary to average (the sum of) many spectra and all n.m.r. spectrometers for biological work have built-in computers for this and other purposes. The sample is placed in a very intense and uniform magnetic field (usually from a superconducting magnet) and surrounded by a coil of wire which both transmits and receives the radiofrequency radiation.

Biological work is best done with pulsed n.m.r., in which short, powerful bursts of radiation at many frequencies are given to the sample. Each resultant signal contains all the information required to generate an entire spectrum by the mathematical process of Fourier transformation but in practice it is usual to add together many signals before transforming them in order to obtain a sufficient signal/noise ratio.

One further aspect of n.m.r. spectroscopy must be mentioned: the time taken for the nuclei perturbed by the radiofrequency signal to relax back to their unperturbed state. This obviously limits the rate at which pulses can usefully be administered to the sample but it also gives information about the local environment of the atom which gives rise to a peak. This is because the perturbed nucleus relaxes by exchanging energy with adjacent magnetic fields. A knowledge of the relaxation parameters of a peak therefore tells much about the molecule in which the nucleus is situated.

Isolated cell preparations

Blood

The experiments of Moon & Richards (1973) illustrated the advantage of the n.m.r. technique. They demonstrated, first, that intra-erythrocytic 2,3-diphosphoglycerate and inorganic phosphate could be detected and measured by $^{31}\text{P}$-n.m.r. and, secondly, that the intra-erythrocytic pH (pH) could be estimated both from the frequency of the 2,3-diphosphoglycerate peaks and also from that of the inorganic phosphate peak. There was good agreement between the two n.m.r.-derived pH values.

This study was extended by Henderson, Costello & Omachi (1974), who were able to detect in addition the three phosphorus peaks of ATP in human erythrocytes. They were also able to demonstrate the increase in 2,3-diphosphoglycerate caused by addition of 10 mmol of inosine/l and 10 mmol of pyruvate/l to aged erythrocytes previously depleted in 2,3-diphosphoglycerate.

Perhaps the most interesting and exciting work on erythrocytes is that of Campbell and his coworkers (Brown, Campbell, Kuchel & Rabenstein, 1977; Brindle, Brown, Campbell, Grathwohl & Kuchel, 1979). These workers have obtained well-resolved proton spectra from erythrocytes by means of a sophisticated pulse technique ('spin-echo') (Campbell, Dobson, Williams & Wright, 1975). The technique takes advantage of the longer transverse relaxation times ($T_2$) of more mobile species in order to select these resonances from the spectrum. Thus they were able to follow the time course of lactate production from glucose and the oxidation-reduction state of glutathione. By measuring the frequency of resonance of the protons on C-2 of the histidine residues of haemoglobin the internal pH could be estimated. In a further study, Brindle et al. (1979) used the spin-echo technique to monitor transport of alanine and lactate into erythrocytes. Although very high concentrations of alanine and lactate were used in order to achieve the required sensitivity the potential of this type of study is considerable. It seems likely that not only can intracellular and extracellular compartments be distinguished by this technique but also compartmentation within the cell.

Tumour cells

Navon, Ogawa, Shulman & Yamane (1977) were able to distinguish the internal pH of Ehrlich ascites tumour cells from the pH of the external medium with $^{31}\text{P}$-n.m.r., a pH difference of 0.46 being obtained. However, after addition of glucose (20 mmol/l) the pH difference fell to zero within 1 h. Various intracellular phosphate-containing metabolites were identified. A further study (Navon, Navon, Shulman & Yamane, 1978) on lymphoid, Friend erythroleukaemic and HeLa cells revealed the absence of a pH gradient in all three cell types but also the presence of an unidentified phosphodiester compound (see also below).
Liver cells

Cohen, Ogawa, Rottenberg, Glynn, Yamane, Brown, Shulman & Williamson (1978) reported 31P spectra of hepatocytes. Two inorganic phosphate resonances were observed which were assigned to the mitochondrial and cytosolic compartments, partly on the basis of their responses to valinomycin, which increased the external pH difference. The lower pH peak, assigned to the cytosol, was sensitive to changes in external pH values; the higher pH was not. It should be noted, however, that this study was carried out at 4°C and although oxygenation was carried out there was very little ATP present in these cells and an inorganic phosphate-free medium was used. Further work on hepatocytes utilizing the 13C-labelled substrates, glycerol (Cohen, Ogawa & Shulman, 1979), glucose and alanine (Cohen et al., 1979; Shulman, Brown, Ugurbil, Ogawa, Cohen & den Hollander, 1979), illustrates the use of this technique for tracing metabolic pathways. With glycerol as substrate, labelling of αglycerophosphate and glucose could be observed. In addition the distribution of 13C in the various carbon atoms of glucose could be detected; initially all the 13C appeared in C-1, C-3, C-4 and C-6 of glucose. This type of study is extremely difficult to achieve by conventional means (e.g. by using 14C) since the labelled products must be separated and then carefully degraded. Exciting though such studies are, they are very expensive to carry out since high concentrations of 13C-labelled substrates must be used (e.g. Shulman’s group used 22 mmol of glycerol/l, 50 mmol of glucose/l and 8 mmol of alanine/l) in order to get a sufficient signal/noise ratio. Much care needs to be taken in interpreting such studies, since dilution with unlabelled endogenous compounds is always possible.

Muscle

Most n.m.r. studies on muscle have utilized isolated, superfused muscles, mainly from amphibians and crustaceans. It is physiologically reasonable to study such small muscles at low temperatures when the rate of oxygen uptake is low enough to be supplied by superfusion. However, early experiments (Hoult, Busby, Gadian, Radda, Richards & Seeley, 1974; Burt, Glonek & Barany, 1976a) utilized ischaemic biopsies. These studies confirmed earlier observations that as phosphocreatine declined in ischaemic muscle the ATP concentration remained relatively constant until the former had almost completely disappeared. Dawson, Gadian & Wilkie (1977) elucidated the changes in inorganic phosphate and phosphocreatine occurring during contraction of frog sartorius muscle and demonstrated a small fall in pH during repeated 25 s tetani, when 20% of the phosphocreatine was hydrolysed. Both Burt et al. (1976a) and Dawson et al. (1977) observed resonances which they could not identify. Seeley, Busby, Gadian, Radda & Richards (1976) and Burt, Glonek & Barany (1976b) identified one of these as glycerol-3-phosphorylcholine. Dawson, Gadian & Wilkie (1978) correlated force development in frog sartorius muscle with changes in phosphocreatine, inorganic phosphate and intracellular pH. They also calculated the rate of ATP hydrolysis and found a strong negative correlation between this and the decline in isometric force development.

Perfused organs

Heart

Garlick, Radda, Seeley & Chance (1977) and Jacobus, Taylor, Hollis & Nunnally (1977) first showed that perfused organs (in this case the rat heart) could be studied by n.m.r. The former group have also investigated the effect of ischaemia and recovery from ischaemia on phosphorus metabolites and intracellular pH with a view to evaluating protective measures against myocardial ischaemic damage (Garlick, Radda & Seeley, 1979).

Kidney

Sehr, Radda, Bore & Sells (1977) studied the effects of ischaemia/reflow cycles on the rat kidney perfused via the circulation of a donor animal. Using 31P-n.m.r. they concluded that the viability of kidneys for transplantation might be assessed by measuring the n.m.r.-derived intracellular pH value, an ability to maintain a relatively high intracellular pH indicating a kidney with minimal ischaemic damage.

Liver

We have been applying 31P-n.m.r. to study the metabolic effects of fructose in the perfused rat liver (Iles, Griffiths, Gadian & Porteous, 1980; Iles, Griffiths, Stevens, Gadian & Porteous, 1980) and with a time resolution of 1–2 min per spectrum we were able to follow the kinetics of the changes in ATP, inorganic phosphate and intracellular pH, which all decline rapidly after infusion of fructose. Similar studies on ischaemia
FIG. 1. Sequence of $^{31}$P-n.m.r. spectra of a perfused (48 h starved) rat liver subjected to a cycle of 30 min ischaemia/30 min reflow. The spectra are recorded either as a function of time after onset of ischaemia (spectra 1–4) or time after subsequent restoration of flow (spectra 5, 6). Peak A arises from several compounds containing phosphorylated sugar residues; peak B from inorganic phosphate; peak C from the $\gamma$-phosphorus nucleus of ATP and $\beta$-phosphorus of ADP; peak D from the $\alpha$-phosphorus of ATP and ADP and the two phosphorus nuclei of NAD; peak E from the $\beta$-phosphorus of ATP. The (intracellular) pH values are derived from the frequency of resonance of peak B (inorganic phosphate).

in starved rats (D. G. Gadian, R. A. Iles, A. N. Stevens, T. R. Griffiths & R. Porteous, unpublished work) have shown that the liver pH appears to decline to a similar extent (by 0.4–0.5) as the pH in glycogen-depleted hearts (Garlick et al., 1979). Fig. 1 shows a series of spectra accumulated during a 30 min ischaemic/30 min reflow cycle in a liver perfusion and illustrates some of the advantages of the n.m.r. technique. Not only can one repeatedly monitor the changes in certain phosphorylated metabolites and intracellular pH during development and recovery from ischaemia, but this can be achieved in a non-destructive manner in one preparation, which is not possible with other more conventional techniques, e.g., Iles, Baron & Cohen (1979).

Two other groups have reported $^{31}$P-n.m.r. studies on perfused livers: McLaughlin, Takeda & Chance (1979) and Salhany, Stohs, Reinke, Pieper & Hassing (1979). Both groups were hampered by the use of narrow-bore n.m.r. spectrometers and needed to accumulate 10–300 times as many spectra to obtain sufficient signal/noise ratios.

Special techniques

Saturation-transfer

This technique has the unique advantage that one can measure the forward and backward rates of enzyme-catalysed reactions in vivo and in vitro (Brown, Ugurbil & Shulman, 1977; Brown, Gadian, Garlick, Radda, Seeley & Styles, 1978) even when the reaction concerned is at equilibrium. It has been applied to both the ATPase reaction in Escherichia coli (Brown et al., 1977) and also the creatine kinase system in frog gastrocnemius and perfused rat hearts (Brown et al., 1978).

Spin-echo

We have already described the spin-echo technique (see above). It might be possible to adapt this method to distinguish metabolites in different compartments within the cell, e.g., mitochondrial, cytosolic and nuclear (Brindle et al., 1979). Several approaches may be adopted. First, the introduction of paramagnetic ions (which contain an unpaired electron) causes signals to be considerably modified due to the generation of a fluctuating magnetic field. Signals (peaks) from a compartment containing such ions may be shifted or be broadened out, often to such a degree that they are not detected. Secondly, the naturally occurring concentration of metal ions which bind to the species under investigation (and alter the resonant frequency of the latter) may be greater in one compartment than another, e.g., magnesium and ATP in mitochondria (Ogawa, Rottenberg, Brown, Shulman, Castillo & Glynn, 1978).

Future potential

One of the most important future n.m.r. applications, as far as the clinician is concerned,
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is the ability to focus on certain defined areas of tissue in a whole organism and thus be able to detect areas (or volumes) of abnormal tissue, e.g., tumours or infarcts. Some progress towards this goal of 'metabolic imaging' has been achieved. It has been common practice for the sample to be enclosed by the radiofrequency coil, which is typically in the shape of a cylinder or saddle. A 'surface coil' design has now been used: this is a 1–4-turn single flat coil which is applied to the surface of the sample (Ackerman, Grove, Wong, Gadian & Radda, 1980). There are two main advantages of this coil design. First, Ackerman et al. (1980) used it to obtain spectra of muscle and brain in an anaesthetized rat, i.e. in vivo. Secondly, some spatial resolution is possible and since the coils can be made extremely small (10 mm diameter) localized volumes of tissue can be scanned. Thus Ackerman et al. (1980) were able to distinguish areas of ischaemic muscle from normally perfused parts. This technique, however, does require surgical exposure of internal organs. In some preliminary work R. E. Gordon, P. E. Hanley, D. Shaw, D. G. Gadian, G. K. Radda, P. Styles & L. Chan (personal communication) have been able to apply static magnetic field gradients to achieve spatial resolution of the liver in an intact anaesthetized rat. This entirely non-invasive 'topical magnetic resonance' would clearly have many uses in medicine if sufficiently large-bore magnets were available.

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References


