ABSTRACTS

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Lecture

Magnetic Resonance Spectroscopy of the Brain

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Magnetic resonance spectroscopy can be used to measure the intracellular concentration of metabolites, and intracellular pH, inside the organs of the body (12). The technique therefore shows great promise for the exploration of metabolic processes, and since it is non-invasive and apparently safe (6) it is particularly suited to the investigation of infants and children.

The phenomenon of magnetic resonance in bulk matter was first described in 1946, idependently by two groups of workers led by Bloch, and Purcell, who as a result of their discovery were awarded the Nobel prize for physics in 1952 (see Andrew (3) for review). Subsequently , magnetic resonance spectroscopy has become very widely used in laboratory physics and chemistry, but it is only in the last few years that studies of living animals and human subjects have become feasible. This development depended on the production of large and powerful superconducting magnets that are necessary for the provision of a sufficiently strong uniform magnetic field (16). The principle of the technique depends on the tendency of certain atomic nuclei with magnetic moments (notably 31P, 1H, 13C, 23Na and 19F), to line up along the field. This alignment can be disturbed by applying a suitable radiofrequency pulse at right angles to the magnetic field. When the pulse ceases, the nuclei return to their previous alignment and in so doing emit a radiofrequency signal which can be detected. The exact frequency of the signal depends on the chemical compound in which the nuclei reside, and the signal-intensity is proportional to concentration. In practice, the part of the body to be examined is normally placed within the horizontal bore of the magnet, and a succession of radiofrequency pulses is transmitted by a surface coil, which also acts as an aerial to detect the signals returning from the tissue (1). These signals are then processed to optimise signal-to-noise ratio. The atomic nucleus most commonly studied so far in the living organism is 31P, largely because the concentrations of phosphorus compounds that are important in energy metabolism can be measured, notably those of adenosine triphosphate (ATP), phosphocreatine (PCr) and inorganic orthophosphate (Pi); moreover, intracellular pH (pH_i) can be calculated from the frequency of the Pi resonance (12). The first tissue to be studied in detail in man, in 1980, was skeletal muscle, because the 20-cm bore of the first suitable magnets was sufficiently large to accommodate a limb. Much new information has been acquired about the energetics of muscle concentration (24), and the technique has also been used to diagnose and investigate disorders of muscle metabolism, including phosphorylase deficiency (McArdle's syndrome) and phosphofructokinase deficiency (11,20).

The brain in newborn infants

The impetus to study newborn infants arose from evidence that the major cause of neurodevelopmental disabilities in infants who require intensive care is cerebral hypoxic-ischaemic injury. Since alterations in the 'high-energy' phosphorus metabolites and in pH_i are to be expected in conditions where oxidative phosphorylation is disrupted, 31P magnetic resonance spectroscopy offered the prospect of exploring the pathogenesis and consequences of hypoxic-ischaemic injury, as well as assessing the efficacy of preventive strategies and treatment. Furthermore, the possibility of obtaining high-resolution spectra from protons (1H), 13C and other atmic nuclei appeared to open up a route towards the non-invasive diagnosis and investigation of inborn errors of metabolism. Since the bore of the available magnet was sufficiently large to admit an adult limb, it seemed likely that a newborn infant could also be successfully studied. Following pilot investigations on experimental animals (10), and the development of a transport system which allowed infants to be studied safely (9), 31P spectra have since 1982 been obtained at the University College London (UCL) from the brains of more than 90 babies, often studied on several occasions (6,17,18). Similar work is under way in Philadelphia (23). The results from UCL may be summarised as follws:

Normal infants. Spectral peaks attributable to ATP, PCr, Pi, and phosphodiesters plus phospholipids were easily detectable from the temporo-parietal cortex of normal infants, together with a large peak resonating in the phosphomonoester region of the spectrum, which is thought to be attributable to phosphoethanolamine (13), a major precursor of membrane phospholipid and myelin. The concentration of this metabolite increased (relative to the other phosphorus metabolites) with decreasing gestational age and fell with increasing postnatal age. Little appears to be present in the adult human brain (5). The concentration of phosphomonoester has been shown in the brain of the newborn rat to fall to adult levels over the first month of life (22). Its presence is therefore clearly related to brain maturation. The mean PCr/Pi ratio in the term infant's brain was 1.35±SD 0.22 (n=6) and mean PCr/ATP was 1.01±0.14 (n=6). These ratios are indices of the phosphorylation potential or 'energy state' of the tissue and were low compared with expected values for adult brain (21); they fell further with decreasing gestational maturity (14). In the rat, the ratios have been shown to increase to adult values over the first weeks of life (22). Mean pH_i in the term infants was 7.14±0.10 (n=6).

Birth asphyxia. Ten term infants who had been severly asphyxiated during delivery were repeatedly studied during the first days of life (17). Intriguingly, the spectra appeared normal or almost so on the first day; but later, PCr fell and Pi increased, causing a fall in PCr/Pi ratio to 0.68±0.34 between the second and ninth days ($p<0.001$ versus normal infants). No other abnormalities were seen in the spectra. When PCr/Pi was minimal, mean pH_i was 7.17 ± 0.10. PCr/Pi subsequently returned towards normal in the surviving infants. Part of the explanation for the low PCr/Pi ratio is likely to be that the intracellular concentration of adenosine diphosphate was raised due to impaired oxidative phosphorylation (17,18). The observation of an apparent latent period after birth before PCr/Pi fell suggests the possibility of effective early treatment in birth asphyxiated infants before irreversible energy failure develops. An animal model of birth asphyxia has therefore been established, in the newborn lamb, so that more can be learnt about the mechanisms of energy failure, and interventions can be tested (19).

Other infants. Infants with a variety of other conditions have been studied (18). Normal spectra were found in, for example, infants with Moebius, Prader-Willi and Pfeiffer syndromes, polycythaemia, mild ventricular distension, and established cerebral atrophy. Abnormal spectra with PCr/Pi ratios below the normal range were detected in infants with increased cerebral echodensities progressing to periventricular leukomalacia and cerebral infarction (14), and in infants with periventricular haemorrhage and post-haemorrhagic hydrocephalus. Two infants with inborn errors of metabolism, propionic acidaemia and arginosuccinic acidaemia, both of which cause serious interference with the tricarboxylic acid cycle, had gross depletion of PCr and ATP, and a profound intracellular acidosis (pH_i 6.41 and 6.45). Little is known of the mechanisms of neurological disturbances associated with inborn errors of amino-acid metabolism. Very recently, it has been shown that the intracellular concentration of one amino-acid, histidine, can be measured by 1H spectroscopy in the brain of the mouse with congenital histidinaemia; measurement of intracellular phenylalanine levels should also shortly become feasible (8). The exploitation of 1H and 13C spectroscopy (2,4) may be expected to make a considerable impact on the elucidation of metabolic abnormalities in the brain and other organs of infants with inborn errors.

Prognosis. Assignment of prognosis is of major concern in neonatal intensive care units. On present evidence, it appears that whatever the diagnosis very low PCr/Pi ratios are incompatible with normal recovery of intracellular metabolism and are associated with a bad prognosis for normal survival (15).

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Workshop

DNA Polymorphism and Detection of Genetic and Infectious Diseases

DNA-Diagnosis of Hemoglobinopathias and Thalassemias

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Hemoglobinopathias and thalassemias are the two major types of inherited disorders of hemoglobin in man. While the hemoglobinopathias exhibit qualitative changes of the globin molecule, the thalassemias result from an imbalance in α- and non-α-globin chain production. In recent years the structural features of the normal human globin genes as well as the molecular lesions in several hemoglobinopathias and many forms of thalassemias have been determined by the application of recombinant DNA technology. While globin gene deletions are the predominant underlying molecular defects in α-thalassemia syndroms, the majority of hemoglobinopathias and β-thalassemias are due to point mutations within the respective globin gene regions.

For diagnostic purposes the identification of mutant genes in cellular DNA is theoretically possible because of the direct or indirect specificity of restriction enzymes. A direct identification of the defective gene can be made if the mutation changed an enzyme's cleavage site and thus changes the normal DNA restriction pattern. For example, the direct detection of the sickle cell gene with restriction enzyme Mst II and the hemoglobin (Hb) M Milwaukee gene with Sst I have recently been described (10,9,2,3,7). An indirect identification of chromosomes that carry a mutant gene relies on the presence of inherited DNA sequence polymorphisms within the cellular genome, giving rise to variations in restriction sites. Examples of this indirect diagnostic procedure are the identification of defective β-globin genes, causing hemoglobinopathias (e.g. Hb Freiburg, Hb Köln, Hb Presbyterian (8,4,5) or β-thalassemias (1,8)).

A third possibility to identify chromosomes carrying point mutations or small deletions relies on oligonucleotide mapping procedures that have successfully been applied for diagnosis of some hemoglobinopathias and thalassemias. Here genotype analysis relies on the detection of normal homozygotes, heterozygotes and defective homozygotes exhibiting the respective three sets of intense, intermediate and missing band signals upon hybridization with oligonucleotides complementary to the normal or the mutated gene sequence. These experimental conditions can also be used in diseases with an autosomal dominant inheritance pattern as in the Hb Freiburg disorder, where normal homozygotes can be differentiated from Hb Freiburg patients (Horst et al. unpublished).

All these methods have been applied for pre- and postnatal diagnostic purposes. In genetic counselling they have been used together with chorion biopsy or amniocentesis to provide prenatal diagnosis in families at risk. In the case of α-thalassemias prenatal diagnosis might only be applied to permit a mother with a fetus with hydrops fetalis to choose whether to carry the fetus through the full 9 month of pregnancy. However, together with hematological and family studies DNA-analysis data are especially useful to differentiate between α-thalassemia-1 and α-thalassemia-2 patients and thus to determine the exact diagnosis (6).

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Molecular genetics of the X-linked muscular dystrophies

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The mutations for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) have been localised to the same region of the short arm of the human X chromosome at Xp21 by linkage analysis to bridging DNA markers (1,2,3). Linkage studies show that the frequency of recombination between markers in this region in the families segregating for these disorders is high (4,5,6). One marker in particular is deleted in both a patient suffering from DMD, chronic granulomatous disease and retinitis pigmentosa (7) and in a patient suffering from DMD and glycerol kinase deficiency (8). The former has a visible cytogenetic deletion. This marker is linked at approximately 10cM from the DMD locus (5,6). An additional marker on the opposite side of the DMD and BMD loci also within Xp21 is linked at a similar genetic distance (9). Although these two markers together can now be used for antenatal diagnosis (10), only a few families can be helped. More closely linked are being identified.

Strategies are now being developed to isolate additional sequences localised within these deletions (11). These approaches should eventually lead to the identification of the molecular basis of DMD and BMD and permit the investigation of the observed high mutation rate and the degree of heterogeneity of the mutations at the DNA level.

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