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In vivo magnetic resonance spectroscopy of human brain: The biophysical basis of dementia

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Abstract

Nuclear magnetic resonance spectroscopy (MRS) in low and medium magnetic fields yields well-resolved natural abundance proton and decoupled phosphorus spectra from small (1–10 cc) volumes of brain in vivo in minutes. With this tool, neurochemical research has advanced through identification and non-invasive assay of specific neuronal — (*N*-acetylaspartate), glial (*myo*-inositol) — markers, energetics and osmolytes, and neurotransmitters (glutamate, GABA). From these simple measurements, several dozen disease states are recognized, including birth injury, and white matter and Alzheimer disease. Addition of stable isotopes of carbon (in man) or nitrogen (in experimental animals) has provided in vivo assays of enzyme flux through glucose transport, glycolysis, TCA-cycle, and the glutamine–glutamate–GABA system. Finally, a number of xenobiotics are recognized with heteronuclear NMR techniques. Together, these tools are having a major impact on neuroscience and clinical medicine. Through diagnosis and therapeutic monitoring, a new generation of in vivo metabolite imaging is expected with the advent of conforming RF coils and higher field NMR systems. © 1997 Elsevier Science B.V.

Keywords: Nuclear magnetic resonance spectroscopy; Neuronal markers; Neurochemistry disorders; Alzheimer disease; Neurotransmitters

1. Introduction

1.1. In vivo MRS in human brain

In this paper, we wish to draw attention to the extensive experience which now exists in performing non-invasive biochemical investigations in man, using nuclear magnetic resonance (NMR = magnetic resonance spectroscopy [MRS] in the biomedical literature).

1.2. Biophysics of human brain disorder

With the advent of NMR, the emphasis of biophysical approaches to neurology has moved from destructive electrophysiology, membrane potential and protein structure, to non-invasive imaging, metabolic mapping and determination of steady-state biochemistry, methods which can be used to explore the intact human brain.

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From the first generation (20 years) of such studies, we have achieved a glimpse of normal and developmental neurochemistry and of some common disorders of neurochemistry which are relevant to our understanding of brain function.

1.3. Methods of brain NMR spectroscopy

NMR detects a frequency dependent signal from individual atomic nuclei, and from populations of such nuclei in living tissues. Spectroscopy is interpreted in terms of identity of the chemical (from chemical shift) and concentration (from the intensity in relative or absolute terms). Spatial information is sacrificed to sensitivity, so that relatively large volumes of tissues or organs give precise chemical information. This contrasts with the sparse chemical information (water only) in microscopic volumes and ultra-short intervals employed in MRI, fMRI and EPI. An intermediate need of information on biological environment is obtained with the same high spatial and temporal resolution, when more subtle NMR-properties (T1, T2, T2*, ρ, etc.) of the highly abundant water molecules are recorded.

These three biophysical techniques are in their infancy to describe human brain function. MRS, the topic of the present talk, has recently completed its first 'descriptive' phase, answering the simple questions: (a) What is normal brain MRS, and (b) what perturbations are encountered in neurologic or psychiatric disease?

1.4. A short history of neurospectroscopy: Milestones in the brain

MRS in the brain began with ^{31}phosphorus spectroscopy in anesthetized rats and other small animals. Non-invasive assays of ATP and phosphocreatine (PCr) (expressed as 'metabolite ratios') and of intracellular pH gave exciting new insights. Direct metabolic rate determination in vivo, using ^{31}P magnetization transfer was among the first biological applications of this now widespread technique.

Global ischemia provided a simple means of testing the methods of MRS and confirming the dependence of cerebral energetics upon oxidative metabolism and glycolysis. A practical future for MRS was demonstrated in the gerbil 'stroke' model, when carotid ligation was clearly shown to produce ipsilateral changes of anaerobic metabolism: Loss of PCr and ATP, increase of inorganic phosphate (Pi) and acidification of the affected hemisphere [1]. It is hard now to realize that prior to those studies, rapid-freezing of whole animals, brain-blowing and surgical biopsy were the only effective source of knowledge of such events.

MRS in the human brain, which began with newborns, quickly verified the predictions of animal studies that hypoxic-ischemic disease of the brain could be monitored by the changes in high-energy phosphates, Pi and pH [2–4]. The predictive value of MRS has been demonstrated in several hundred newborn infants. The outcome after severe hypoxic ischemic encephalopathy in newborn humans is determined by the intracerebral pH and Pi/ATP ratio.

Wide-bore high-field magnets (from Oxford Magnet Technology) permitted extension to adults and infants beyond a few weeks of age [5]. Three areas of human neuropathology have as a result been extensively illuminated by ^{31}P-MRS. Using brain tumor as a target of newly evolving localization techniques (the early studies in infants employed no localization beyond that conferred by the 'surface coil') Oberhaensli et al. began the slow process of overturning three decades of thought about brain tumors in particular, showing their intracellular pH to be generally alkaline not acidic [6]. A new generation of drugs in oncology will be designed to enter alkaline intracellular environments, rather than the acidic environment measured in the interstitial fluid.

^{31}P-MRS in adult stroke exactly mirrored the findings in hypoxic-ischemic disease of newborns, and even appears to offer predictive value through intracellular pH and Pi/ATP [7]. This work in turn has provoked a large body of research in experimental models of stroke which now guides the human application of MRS.

The third area of work stimulated by the advent of ^{31}P-MRS was that of degenerative disease of the brain, including Alzheimer disease [8]. Commencing with in vitro studies of tissue extracts, two hitherto unrecognized groups of compounds, seen in the ^{31}P spectrum as phosphomonoesters (PME) and phosphodiesters (PDE), were empirically shown to be altered. Much of the work was unsatisfying, since unlike ATP, PCr and Pi, the identity and metabolic

significance of these 'peaks' was incompletely understood. Nevertheless, a promising new area of neurochemistry was opened by ^{31}P-MRS, and then extended to in vivo brain analysis in patients. Modern Neurospectroscopy is much more like this than was anticipated by the earlier investigators. What MRS has done in neurochemistry, is what virtually all spectroscopic techniques have done in their time. By providing non-invasive assays of less well known metabolites and pathways, MRS has identified a 'New Neurochemistry.' A perfect example of this new knowledge emerged with the advent of water suppressed ^{1}H-MRS of the brain in vivo Fig. 1.

N-acetylaspartate (NAA), a neuronal marker [9], was re-discovered in 1983 [10]. Of the many expected and new resonances now identified in clinical practice of neuro-MRS, none has created more diagnostic information than NAA. Its identity, concentration and distribution are now well established. Early experiments in animals showed loss of NAA in stroke. Very large numbers of studies in man show NAA absent, or reduced in brain tumor (glioma), ischemia, degenerative disease, inborn-errors and trauma, so that to a first approximation, the histochemical identification of NAA [and *N*-acetyl aspartyl glutamate (NAAG)] with neurones and axons, and its absence from mature glial cells, is confirmed. The clinical use of ^{1}H-MRS as an assay of neuronal 'number' is justified. In the ten years since its introduction, 50–100 clinical applications of this assay have emerged and placed neurospectroscopy far ahead in the introduction of MRS into routine clinical practice [11].

The first generation of MRS studies were performed without image-guidance. While MRI is not essential to our understanding of neurochemistry, the combined use of these two powerful tools permitted the direct demonstration that there is often a dissociation in space, between anatomically obvious events in the brain and biochemical changes. Metabolite

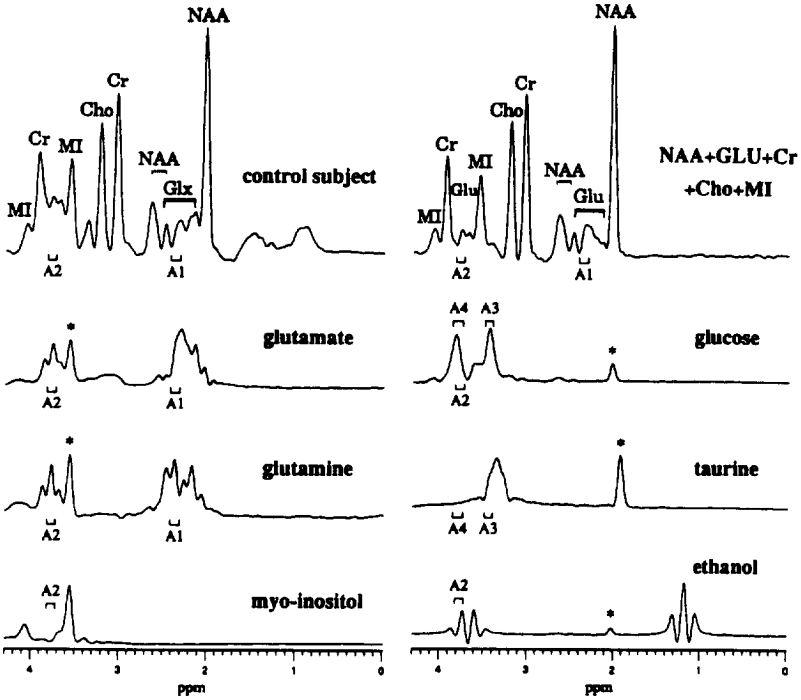

Fig. 1. Human brain proton MRS. The spectrum of human brain in vivo is illustrated (top left). It is faithfully reproduced in the model solution (top right) which is composed of the individual metabolites indicated. Spectra of common metabolites acquired under similar conditions as also shown. Acquisition: GE 1.5 T; STEAM TE 30 TR 1500.

imaging has confirmed this important principle in stroke, tumors, multiple sclerosis and degenerative diseases.

1.4.1. Dissociation in time

The other side of the same coin is the realization that abnormalities in the anatomy, as revealed by MRI, are not *essential* to the detection of biochemical disorders by MRS. A simplified method of localization permitted the routine use of MRS to assay neurochemistry in a single place, albeit rather large, in the cerebral cortex, cerebellum or mid-brain. This method, now generally known as 'single-voxel MRS' was pioneered by Frahm and co-workers and by Shulman and co-workers and is largely responsible for showing that biochemical disorders commonly underlie neurological disease [12–19] MRS is therefore well poised for 'early' diagnosis.

MRS accurately reflects developmental changes in normal infant brain. Reversible biochemical changes accompany systemic physiological events. In addition to the inborn-errors of metabolism and hereditary diseases, which are relatively rare, several of the major neurological scourges of our time are identified by their neuro-MRS patterns: Neonatal hypoxia, cerebral palsy, neuro-AIDS, dementias, stroke, epilepsies, neuro-infections and many encephalopathies are now seen to include a biochemical component. Often this can only be diagnosed with MRS.

1.4.2. Automation and quantitation

Even when MRI identifies disease very accurately and very early, there is a strong possibility that MRS could do so earlier, and therefore increase the window of therapeutic opportunity so much needed in neurology. Automation permits universal access, including urgent MRS in acute, reversible neurological diseases, and large scale clinical trials. Quantitation, a long-overlooked area, gives the precision of measurement which will be required to conclusively demonstrate incremental metabolic responses to intervention and therapy.

1.4.3. ^{31}P-, ^{1}H–^{31}P-, ^{13}C-, ^{1}H–^{13}C- and ^{15}N-MRS

A parallel technical development of MRS with other nuclei, either directly detected or, to achieve greater sensitivity, observed through the proton signal, although generally less readily applied in clinical settings has already yielded many interesting physiological findings. It is now time to explore their value in clinical diagnosis again, using information gained from several years of ^{1}H-MRS.

2. Proton MRS in Alzheimer disease

The proton spectrum in AD is startlingly different from normal, with elevated mI/Cr and reduced NAA/Cr Fig. 2 [20–23]. This pair of abnormalities distinguishes AD from normal elderly, while mI/Cr distinguishes most AD from other dementias, with the possible exception of frontal lobe dementia (FLD, or Pick"s disease). Common, and *therefore confounding, metabolic disorders* which also influence cerebral mI/Cr and [mI] are: Renal failure [24], diabetes mellitus [25], chronic hypoxic encephalopathy (unpublished) and hypernatremia [26], all of which have increased mI. Hepatic encephalopathy [27,28], including subclinical hepatic encephalopathy [29], and hyponatremia [30] result in decreased cerebral mI concentration.

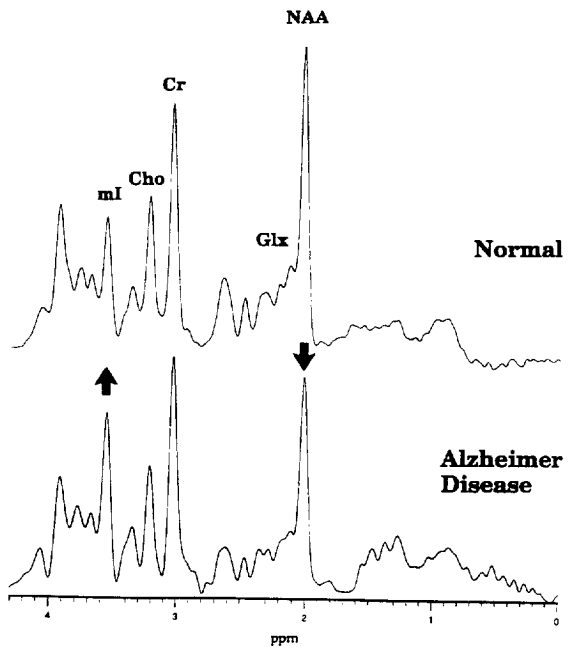

Fig. 2. Abnormalities of ^{1}H-MRS in a patient with Alzheimer disease.

3. Rapid screening for AD using automated ^{1}H-MRS (PROBE)

A useful capability in screening large numbers of patients for MRS trials will be a 7–10 min automated (but non-quantitative) ^{1}H-MRS examination which has shown itself robust and accurate [31], and can be added to a normal imaging examination at sites other than HMRI [32].

4. The biochemical basis of Alzheimer disease

There are currently three major biochemical theories for the etiology of AD (in addition to the many suggestive studies for a non-metabolic etiology), to which we propose to add a fourth. **The beta-amyloid theory,** summarized most recently by Selkoe [33] proposes that this long recognized abnormal protein of Alzheimer diseased human brain is neurotoxic. Symptoms are associated with the later appearing neurofibrillary plaques and tangles. **The cholinergic hypothesis** most associated with Wurtman, identified early loss of cholinergic neurons, particularly in the hippocampal gyrus of the temporal lobe, and alterations in membrane phospholipids particularly of the choline family [34] again principally but not exclusively from the temporal lobe with the highly specific neurobehavioral deficits of AD. Post-mortem studies have identified what are presumably related alterations in the phosphoryl choline and glycerophosphoryl choline concentrations which can therefore provide in vivo detectable correlates through the use of ^{31}P-MRS to identify phosphomonoester (PME) and phosphodiester (PDE) peaks, and to identify with ^{1}H-MRS the sum of these as the 'Cho' peak. **The APOE theory**, is the latest and most universally applicable of **the genetic hypotheses**. In addition to the circa 10% of familial, early onset AD which has been previously identified with specific chromosome abnormalities (Chromosome-21 in Down syndrome is the most frequent example), Apolipoprotein E is the clearest demonstration to date that identifies a gene conferring increased risk of AD in the general population (most recently reviewed in relation to the Amyloid theory [35]). ApoE e4 is about 3 times over-represented in late onset AD [36].

These three theories are not mutually exclusive, although the connection between them is not at all clear [37]. Indeed they are mutually supportive leading to important areas of research and a rational therapy. At the present time empirical therapy is available to test only one of these three theories, that based upon the 'cholinergic' hypothesis for which the drugs tacrine and Aricep were designed. However, the early trials of tacrine have been of limited success, so that they do not conclusively support the cholinergic hypothesis, and furthermore leave open the mode of action of Tacrine in those patients in whom there is a clinical response [38]. Cholinergic receptors in the central nervous system are believed in part to act through the phosphoinositide pathway [39,40]. Thus, the cholinergic hypothesis could be considered in one sense, an extension of the **inositol hypothesis**.

The inositol hypothesis of Alzheimer Disease, as we would suggest, is based upon three indepen-

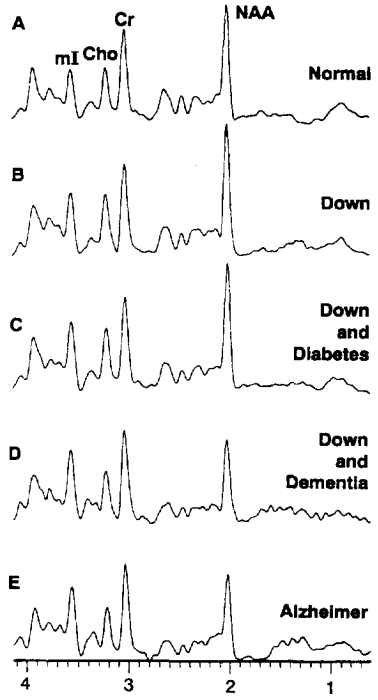

Fig. 3. Elevated cerebral *myo*-inositol, an early finding in Down Syndrome. Representative ^{1}H-NMR spectra of adults with Down Syndrome showing elevated mI, and Cho. NAA remains normal in Down's patients until dementia occurs. At this stage ^{1}H-MRS is indistinguishable from true Alzheimer disease (lower two spectra).

dent lines of experimental evidence. In historical order, they are:

(1) Stokes and Hawthorne [41] identified a marked depletion of the membrane lipid phosphatidyl inositol in post mortem AD brain. They postulated defective biosynthesis of phosphatidyl inositol.

(2) Jolles et al. [42] showed a reduced activity of the inositol polyphosphate (IPP) enzyme phos-

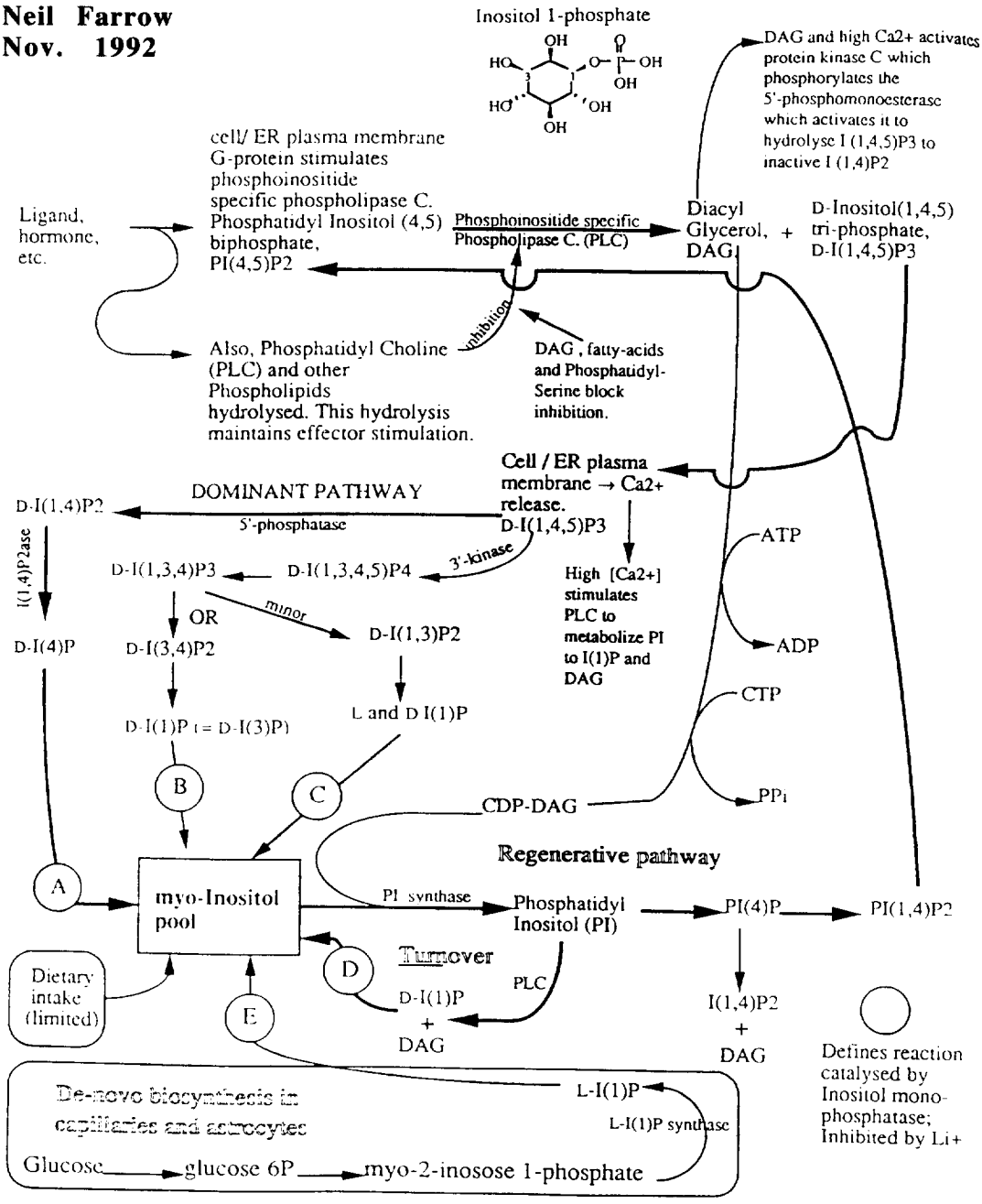

Fig. 4. Schematic of cerebral inositol phosphate metabolism.

phatidylinositol kinase and postulated a specific defect of the inositol polyphosphate cascade in AD brain, such that cholinergic activity could be impaired.

(3) Miller et al. [20]; Shonk et al. [23]; Moats and Shonk [31]; and Moats et al. [43]; identified a 20–30% increase in the concentration of '*myo*-inositol' (the polyol at highest concentration of any of the inositol metabolites in brain), in mild to moderate AD, as opposed to other dementias [31]. Shonk and Ross [44], using ^{1}H-MRS in adults with Down syndrome observed that significant elevation of mI occurs **before the onset of dementia** and without any concomitant reduction in the neuronal marker NAA. Furthermore, in a single patient with both Down syndrome and dementia, the MR spectrum was indistinguishable from that of AD patients, with elevated mI and reduced NAA concentrations (Fig. 3). These findings have recently been confirmed by Shapiro and colleages (unpublished 1997) at NIH.

Because ^{1}H-MRS alone cannot distinguish between free inositol and its major phosphorylated precursor, inositol-1-phosphate (IP), it is probably more accurate to refer to the observations above as an increase in inositols, *myo*-inositol **plus** inositol-1-phosphate, although Stokes and Hawthorne also observed increase in the concentration of mI itself in several AD brains examined post-mortem. Furthermore the preponderance of mI over inositol-1-phosphate in normal brain is more than 10 to 1.

5. Cause(s) of increased mI in probable AD?

Pathways of cerebral inositol metabolism are extremely complex and incompletely known (Fig. 4). While the major site of mI biosynthesis may actually be outside the brain, all of the pathways shown are represented within the brain. Most of the individual intermediates shown in Fig. 4 are present briefly or at very low concentrations, not detectable with in vivo MRS, while the major membrane phospholipid phosphatidylinositol (PI) is relatively immobile and also likely to be MR-invisible. Nevertheless, at least five independent mechanisms can be postulated for the observed increase in cerebral mI:

(1) A specific metabolic marker of one of the known histological events of AD: Amyloid, plaques or tangles [35,45–48].

(2) A marker for cell types rich in mI, which replace neurons in AD brain. This would apply to glial cells; hence mI may mark regions of 'gliosis' [49].

(3) Enhanced activity of a sodium: mI transporter in the (probably glial) cell membrane, resulting in a higher steady-state concentration of mI. This mechanism is postulated for cultured fibroblasts of Down syndrome, but was *NOT found in AD* [50,51].

(4) Inhibition of enzymic conversion of mI to phosphatidylinositol (PI) would explain the observation of reduced PI in post-mortem AD brain, even though no significant increase in mI was found in that study [41].

(5) Excessive activity of inositol monophosphatase in early AD (and possibly Down syndrome also), could result in conversion of IP to mI. This now appears to be the most intensely regulated enzyme of the IPP cascade, and following its recent crystallization and NMR structural analysis is the leading candidate for the non-competitive *inhibition of inositol metabolism by lithium* [52–58].

Much of the recent evidence for an *inositol hypothesis* depends upon ^{1}H-MRS. In normal brain the 'peak' contains contributions from the equilibrium concentrations of mI:IP which are 5 mM:0.5 mM,

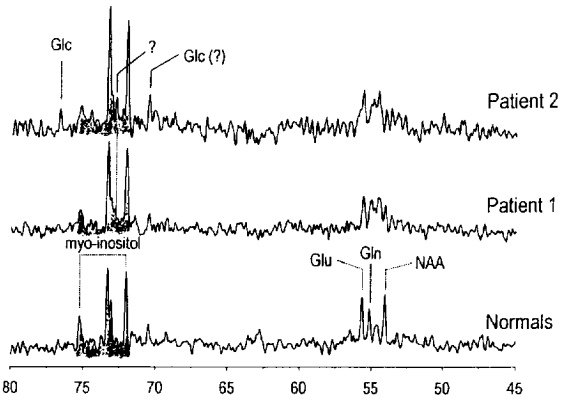

Fig. 5. Natural abundance ^{13}C-MRS at 4.1 T in two patients with Alzheimer disease.

together with 0.5–1.0 mM glycine, and underlying metabolites which may contribute up to 25% of the entire peak area [59]. Because of the remaining uncertainty of the identity of the 3.58 ppm peak in the proton spectrum in AD, alternative interpretations abound.

6. Localization and natural abundance ^{13}C-MRS of AD

^{13}C-MRS demonstrates that increased *myo*-inositol is present in the brain of two patients with probable Alzheimer disease. Fig. 5 illustrates the

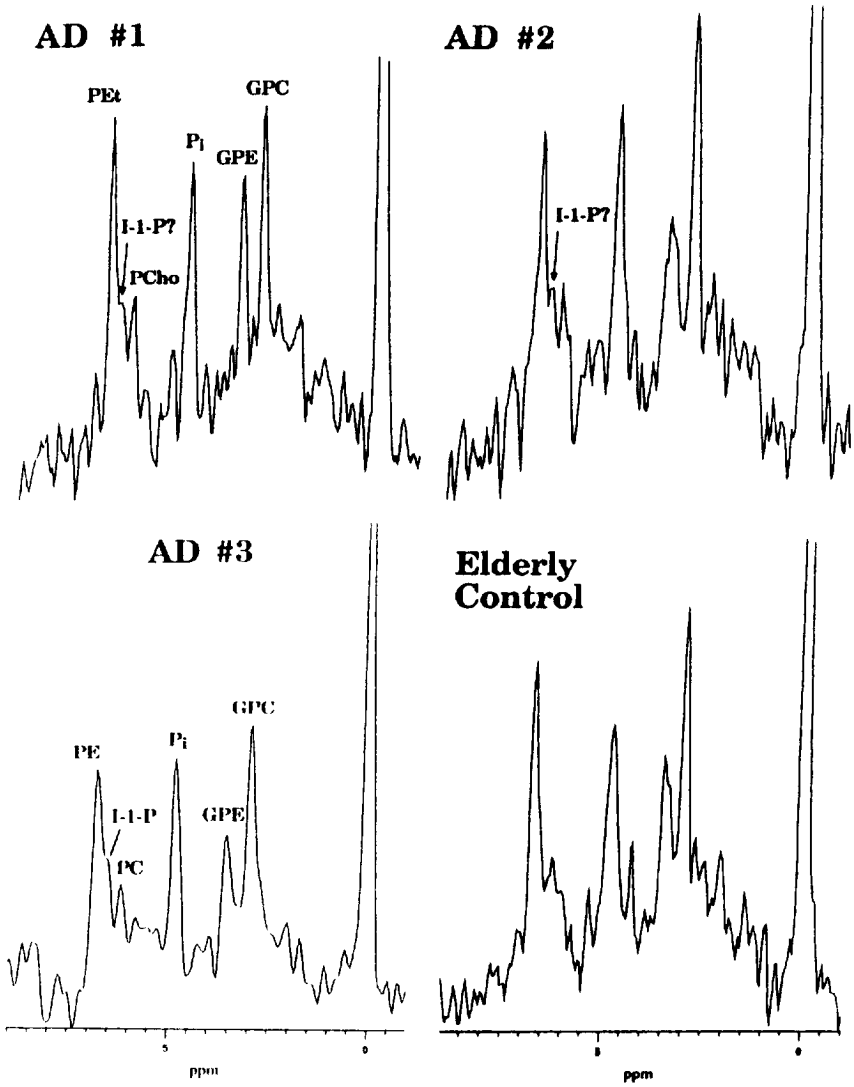

Fig. 6. Proton decoupled ^{31}P-MRS in three patients with Alzheimer disease. Note the excellent resolution of mono-esters and diesters. There is no obvious abnormality of the choline metabolites PC and GPC, in these in vivo spectra, suggesting that earlier reports may be the result of post mortem changes. Note: The assignment of I-1-P suggested in this figure is likely to be erroneous, based on new studies with model solutions (Fig. 7).

striking results of a collaboration between Gruetter (University of Minnesota) and the MRS Group of HMRI [60].

7. Possible consequences of elevated mI in AD: Benefits of reversal or prevention?

Assuming, as seems reasonable that it is an increase in the dominant metabolite *myo*-inositol which is being observed in the brain of patients with probable AD we may reasonably ask how this might contribute to the disease? It seems unlikely that mI is toxic, and apart from a suggested association between *myo*-inositol **depletion** and visuopractic dysfunction in patients with subclinical hepatic encephalopathy [29], no specific neuropsychological function has ever been ascribed to *myo*-inositol itself. Thus, it is not possible at present to relate this neurochemical change to the clinical picture of AD. On the other hand, altered [mI] must have consequences for the enzyme equilibria and metabolite concentrations in the inositol polyphosphate cascade. We could predict altered hormone sensitivity of the AD brain (some 15–20 hormone receptor mechanisms depend upon the IPP receptor system) or altered cholinergic sensitivity (a possible link with the current choline hypothesis of AD), altered local osmotic disequilibrium or altered mI as an epiphenomenon to the overall process of AD.

Modifying cerebral [mI], if it were possible, might have a larger or smaller effect on AD and its progression. At one extreme, we could postulate that preventing or reversing the accumulation of mI in the AD brain might conserve neuronal function by inhibiting the (secondary) loss of NAA, the neuronal marker. At the other extreme, major shifts in mI, inositol phosphate and the equilibrium in the IPP cascade could be entirely without effect in established AD, even when diagnosed early. Conversely, it may be postulated that a drug, such as Tacrine which (sometimes) has beneficial effects in AD has hitherto unsuspected effects on mI metabolism.

Exploring whether mI and/or IP can be altered in established AD could shed some much-needed light on this newly recognized and potentially very complex biochemical disorder. In the absence of an appropriate animal model, and with the advantages of new and reliable quantitative MRS in vivo, such a study is timely in man.

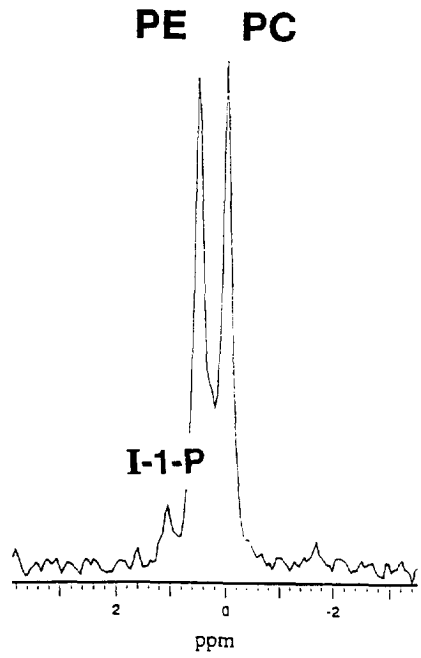

Fig. 7. In vitro spectrum of phospho-mono-esters at 1.5 T. Inositol-1-phosphate appears as a distinct resonance **not** between phosphoethanolamine and phosphocholine.

Quantitative, ^{31}P-MRS ± proton decoupling [61–64] has now been performed in a small number of patients with Alzheimer disease. Initial results fail to show any systematic effect on PC or GPC (Fig. 6). Furthermore, initial suggestions of increased inositol-1-phosphate in AD [63,64] must be treated with caution, as the actual chemical shift of this intermediate is still controversial (Fig. 7).

8. Future biomedical studies with NMR in brain

A striking feature of all in vivo MRS studies is the novelty of the physio-chemical information obtained. Thus, some critical features of the disease process may be lost when the standard tissue extraction of preparation techniques are applied.

Conversely, the nature and quality of the NMR techniques which are currently available for such studies in human brain in vivo is sadly constricted,

when compared to the versatility of in vitro physiochemical methods already evolved from physical NMR laboratories around the world. Much is to be gained therefore by exploring methods which extend to biology, the power of in vitro NMR. (An intermediate path commonly employed in clinical research is the development of in vitro models of the pathological process: Either in experimental animals, in tissue cultures, or in artificially synthesized membranes or cellular chemicals).

An immediate solution, offered in this brief survey of a modern epidemic-Alzheimer disease, for which no entirely satisfactory animal model exists, is the use of ultra-high field (4 T and upward) whole body magnets.

A second, is the use of more hetero-nuclear NMR techniques, commonly used to probe protein and macro-molecular structure. Here ^{15}N- and $^{1}H-^{15}N$-NMR has shown considerable promise in one neurological condition, hepatic encephalopathy [65], but has yet to be applied to the complex problem of Alzheimer disease.

A third is the development of solid-state NMR methods of membrane analysis to these sophisticated in vivo questions. Currently, virtually all such methods are excluded by the need to spin samples. Novel gradient methods combined with high fields offer some promise.

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