

## Proton and 31-phosphorus neurospectroscopy in the study of membrane phospholipids and fatty acid intervention in schizophrenia, depression, chronic fatigue syndrome (myalgic encephalomyelitis) and dyslexia

BASANT K. PURI

*MRC Clinical Sciences Centre, Imperial College, Hammersmith Hospital, London, UK*

### Summary

Neurospectroscopy allows biochemical processes in the brain to be studied non-invasively. At magnetic field strengths of 1.5 T or higher, cerebral proton neurospectroscopy allows the ascertainment of values of myo-inositol, choline-containing compounds, creatine, glutamate, glutamine, and N-acetyl aspartate. At similar field strengths, cerebral 31-phosphorus neurospectroscopy allows the ascertainment of values of phosphomonoesters, inorganic phosphate, phosphodiester, phosphocreatine, and the gamma, alpha and beta nucleotide triphosphate (mainly adenosine triphosphate) resonances. Since choline is a common polar head group at the Sn3 position of membrane phospholipid molecules, a raised level of free choline, as indexed by proton neurospectroscopy, can indicate relatively low anabolism of membrane phospholipid molecules. Furthermore, the choline peak includes phosphorylcholine and glycerophosphorylcholine and even ethanolamine. The phosphomonoesters peak measured using 31-phosphorus spectroscopy includes major contributions from phosphocholine, phosphoethanolamine and L-phosphoserine, which are important precursors of membrane phospholipids, while the phosphodiester peak includes contributions from glycerophosphocholine and glycerophosphoethanolamine, which are important products of membrane phospholipid catabolism. Hence proton neurospectroscopy and 31-phosphorus neurospectroscopy can yield important information relating to the metabolism of cerebral membrane phospholipids. The application of these techniques to the investigation of membrane phospholipid metabolism in schizophrenia, depression, chronic fatigue syndrome (myalgic encephalomyelitis or M.E.) and dyslexia is described.

### Introduction

Magnetic resonance imaging scanners with magnetic field strengths of 1.5 T or higher are now readily available in most leading medical centres. They are mainly used for structural imaging purposes at the current time. Since the proton resonance frequency (63.7 MHz at 1.5 T) is used to acquire data for structural imaging, it is not too difficult, using appropriate software, to carry out proton neurospectroscopy using these scanners. 31-Phosphorus spectroscopy involves measuring signals from 31-phosphorus-containing molecules at a lower frequency (25.8 MHz at 1.5 T), and so a different coil is needed within the magnet. While 31-phosphorus neurospectroscopy allows determination of the ratio of membrane phospholipid anabolism to catabolism, even without access to the extra hardware and software needed for this type of spectroscopy the more readily available proton neurospectroscopy can yield valuable information relating to membrane phospholipids.

After describing these two methods, their application to studying membrane phospholipid metabolism in relation to fatty acids in a number of disorders is described.

### Proton neurospectroscopy

*In vivo* proton neurospectroscopy studies are associated with a number of technical challenges, including: the water signal is around ten thousand times larger than signals from metabolites of interest, and so needs to be suppressed; the chemical shift (which describes the relative frequency position of a peak resonance) covers a narrow range so that peak overlap is a problem and there are stringent demands on magnetic field homogeneity; interaction between nearby protons within a molecule (spin–spin coupling) complicates the spectral pattern; the lipid signal from the scalp is much larger than the metabolite signals of interest from the underlying brain (Cox & Puri, 2004). Notwithstanding these difficulties,

at magnetic field strengths of 1.5 T or higher, cerebral proton neurospectroscopy allows the ascertainment of peaks corresponding to resonances for myo-inositol, choline-containing compounds, creatine, glutamate, glutamine, and N-acetyl aspartate. Of these, the choline peak is of particular interest in relation to phospholipid metabolism. This peak contains contributions from choline, phosphorylcholine, glycerophosphorylcholine, and even a small amount of ethanolamine. Choline is one of the common polar head groups attached at the Sn3 position of membrane phospholipid molecules. Phosphorylcholine is involved in membrane phospholipid anabolism, as well as acting as a second messenger (for example for mitogenic activity of growth factors; Cuadrado, Carnero, Dolfi, Jimenez and Lacal, 1993). Glycerophosphorylcholine is involved in membrane phospholipid catabolism. Ethanolamine, like choline, is a polar head group often attached to phospholipids. At current magnetic field strengths in clinical use, it is extremely difficult to differentiate between the relative contributions of phosphorylcholine and glycerophosphorylcholine, without recourse additionally to 31-phosphorus neurospectroscopy and its phosphomonoester and phosphodiester peaks.

### 31-phosphorus neurospectroscopy

At magnetic field strengths of 1.5 T or higher, cerebral 31-phosphorus neurospectroscopy allows the ascertainment of peaks corresponding to resonances for phosphomonoesters, inorganic phosphate, phosphodiester, phosphocreatine, and gamma, alpha and beta nucleotide triphosphate. The first and third of these peaks are important in respect of membrane phospholipid metabolism. The phosphomonoester peak reflects membrane phospholipid anabolism. This is because it contains contributions from freely mobile phosphomonoesters, including phosphocholine, phosphoethanolamine and even small contributions from inositol phosphate, glycerophosphate, phosphothreonine and L-phosphoserine, and also contributions from less mobile phosphomonoester-containing molecules, such as certain phosphorylated proteins, and from certain proteins that are part of the neuronal cytoskeleton. In contrast, the phosphodiester peak reflects membrane phospholipid catabolism as it contains contributions from freely mobile phosphodiester, including glycerophosphocholine and glycerophosphoethanolamine. The phosphodiester peak also contains contributions from less mobile phosphodiester-containing molecules, such as molecules involved in membrane structure (both cell membranes and intracellular organelle membranes). In addition, there is a broad peak present which corresponds to

mobile phospholipids, such as those occurring in structures such as vesicles.

### Schizophrenia

In a pilot study of membrane phospholipid metabolism in first-episode drug-naïve patients with schizophrenia, Pettegrew et al. (1991), used 31-phosphorus neurospectroscopy to study the dorsal prefrontal cortex of 11 patients and 10 healthy control volunteers matched with respect to age, education and parental education. The patient group had significantly lower levels of phosphomonoesters and inorganic phosphate and significantly higher levels of phosphodiester and nucleotide triphosphate. Whilst the nucleotide triphosphate and inorganic phosphate findings suggested functional hypoactivity of the dorsal prefrontal cortex in this patient group, the findings regarding phosphomonoesters and phosphodiester indicated alterations in membrane phospholipid metabolism in these patients, with reduced biosynthesis of and increased breakdown of neuronal membrane phospholipids. These findings were consistent with the late Professor David F. Horrobin's membrane phospholipid model of schizophrenia (see Horrobin & Bennett, 2003). The findings from subsequent studies have not been consistent, however (Puri, 2000).

### Depression

The first published case of a patient with depression to be treated with eicosapentaenoic acid (EPA; Puri, Counsell, Hamilton, Richardson, & Horrobin, 2002a) underwent 31-phosphorus neurospectroscopy both at baseline and after nine months of taking this fatty acid. This was a severe case of treatment-resistant depression in a 21-year-old male student with a seven-year history of unremitting depressive symptoms. After the addition of ultra-pure EPA (in the form of the ethyl ester) there was rapid clinical improvement, including the cessation within one month of previously unremitting severe suicidal ideation; there was also a marked improvement in social phobia symptomatology. There was progressive clinical improvement, and by nine months the patient's depressive symptoms had disappeared altogether, without any adverse effects from the EPA. During the nine-month period of treatment with EPA, there was a 53% increase in the relative phosphomonoester peak value (expressed as a ratio to the total 31-phosphorus signal) and a 10% decrease in the relative phosphodiester peak value (Puri, Counsell, Hamilton, Richardson, & Horrobin, 2001). These results, taken together with a 30% increase in the volumetric niacin response over the same time, indicate that EPA supplementation may

have been associated with reduced neuronal phospholipid turnover, with a large increase in cerebral phospholipid biosynthesis and some decrease in phospholipid breakdown (Puri, 2003).

### Chronic fatigue syndrome

The first systematic neurospectroscopy study of chronic fatigue syndrome (or myalgic encephalomyelitis, M.E.) was that of Puri et al., (2002b), in a comparison of eight patients with eight age- and sex-matched healthy control subjects. Using proton neurospectroscopy, it was found that the mean ratio of choline to creatine in the occipital cortex was significantly higher in the patients than in the controls, indicating an increase in the choline peak, as creatine is considered to be relatively stable. In addition, there was a loss of the normal spatial variation of the choline peak in the chronic fatigue syndrome patients. The second systematic neurospectroscopy study, by Chaudhuri, Condon, Gow, Brennan and Hadley, (2003), also used proton spectroscopy in eight chronic fatigue syndrome patients and also found a higher choline resonance in the patients compared with age- and sex-matched healthy controls. Finally, in a previous proton neurospectroscopy report of three children, aged 11, 12 and 13 years, with chronic fatigue syndrome, Tomoda et al., (2000) also found elevation of the ratio of choline to creatine. Since choline is often a polar head group in the phospholipid molecule, these results are consistent with the hypothesis that there is an abnormality of membrane phospholipid metabolism in chronic fatigue syndrome (Puri, 2005), which in turn may be the result of a persistent viral infection.

### Dyslexia

The first systematic 31-phosphorus neurospectroscopy study of dyslexia was carried out by our group (Richardson, Cox, Sargentoni, & Puri, 1997) in 12 dyslexic and 10 non-dyslexic adults. The phosphomonoester peak area was significantly elevated in the dyslexic group, compared with the controls. These findings were consistent with reduced incorporation of phospholipids into cell membranes. Interestingly, a membrane phospholipid model of dyslexia, in which the phosphomonoesters would be raised rather than reduced, as in schizophrenia, had previously been suggested and was supported by these findings (see Puri & Richardson, 2003).

### Discussion and conclusions

It has been shown that proton neurospectroscopy and 31-phosphorus neurospectroscopy can be successfully applied to the study of membrane

phospholipid metabolism in adults suffering from disorders such as schizophrenia, depression, chronic fatigue syndrome and dyslexia.

### References

- Chaudhuri, A., Condon, B. R., Gow, J. W., Brennan, D., & Hadley, D. M. (2003). Proton magnetic resonance spectroscopy of basal ganglia in chronic fatigue syndrome. *NeuroReport*, *14*, 22–228.
- Cox, I. J., & Puri, B. K. (2004). *In vivo* MR spectroscopy in diagnosis and research of neuropsychiatric disorders. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *70*, 357–360.
- Cuadrado, A., Carnero, A., Dolfi, F., Jimenez, B., & Lacal, J. C. (1993). Phosphorylcholine: A novel second messenger essential for mitogenic activity of growth factors. *Oncogene*, *8*, 2959–2968.
- Horrobin, D. F., & Bennett, C. N. (2003). Phospholipid metabolism and the pathophysiology of psychiatric and neurological disorders. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 3–47). Carnforth: Marius Press.
- Pettegrew, J. W., Keshavan, M. S., Panchalingam, K., Strychor, S., Kaplan, D. B., Tretta, M. G., & Allen, M. (1991). Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naive schizophrenics. A pilot study of the dorsal prefrontal cortex by *in vivo* phosphorus-31 nuclear magnetic resonance spectroscopy. *Archives of General Psychiatry*, *48*, 563–568.
- Puri, B. K. (2000). MRI and MRS in neuropsychiatry. In I. R. Young, D. M. Grant & R. K. Harris (Eds.), *Methods in Biomedical Magnetic Resonance Imaging and Spectroscopy* (pp. 1135–1143). Chichester: Wiley.
- Puri, B. K. (2003). A possible role for fatty acid treatment in paediatric depression. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 543–548). Carnforth: Marius Press.
- Puri, B. K. (2005). *Chronic fatigue syndrome*. London: Hammersmith Press.
- Puri, B. K., & Richardson, A. J. (2003). Brain phospholipid metabolism in dyslexia assessed by magnetic resonance spectroscopy. In M. Peet, I. Glen & D. F. Horrobin (Eds.), *Phospholipid Spectrum Disorders in Psychiatry and Neurology* (pp. 501–508). Carnforth: Marius Press.
- Puri, B. K., Counsell, S. J., Hamilton, G., Richardson, A. J., & Horrobin, D. F. (2001). Eicosapentaenoic acid treatment in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover. *International Journal of Clinical Practice*, *55*, 560–563.
- Puri, B. K., Counsell, S. J., Richardson, A. J., & Horrobin, D. F. (2002a). Eicosapentaenoic acid in treatment-resistant depression. *Archives of General Psychiatry*, *59*, 91–92.
- Puri, B. K., Counsell, S. J., Zaman, R., Main, J., Collins, A. G., Hajnal, J. V., & Davey, N. J. (2002b). Relative increase in choline in the occipital cortex in chronic fatigue syndrome. *Acta Psychiatrica Scandinavica*, *106*, 224–226.
- Richardson, A. J., Cox, I. J., Sargentoni, J., & Puri, B. K. (1997). Abnormal cerebral phospholipid metabolism in dyslexia indicated by phosphorus-31 magnetic resonance spectroscopy. *NMR in Biomedicine*, *10*, 309–314.
- Tomoda, A., Miike, T., Yamada, E., Honda, H., Moroi, T., Ogawa, M., Ohtani, Y., & Morishita, S. (2000). Chronic fatigue syndrome in childhood. *Brain and Development*, *22*, 60–64.