Muscle high-energy phosphates in central nervous system disorders.
The phosphorus MRS experience

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Phosphorus magnetic resonance spectroscopy (MRS) was used to study muscle phosphates metabolism in several brain disorders. Those with primary mitochondrial encephalomyopathies showed the typical pattern of impaired oxidative metabolism at rest and during recovery after exercise. In migraine, Parkinson’s disease and alternating hemiplegia muscle MRS observations lend support to a possible mitochondrial dysfunction. Similar observations in multiple sclerosis are probably the result of secondary deconditioning. In post polio syndrome and in some of the hereditary ataxias, elevated intracellular inorganic phosphates may be the result of another, yet unknown, metabolic impairment. Thus, muscle phosphate metabolism may be altered in various central nervous system (CNS) disorders by different metabolic impairments. All these possibilities should be taken into account when evaluating MRS results in brain diseases.

Key Words: Muscle — Phosphorus MRS — Spectroscopy — CNS disorders.

Introduction

Phosphorus magnetic resonance spectroscopy (MRS) is a particularly suitable noninvasive technique for detecting intracellular phosphates in muscle. Over the last 10 years, we and others have been using this technique to study the metabolism of muscle high-energy phosphates in various neurological disorders. Muscle phosphorus MR spectrum contains five major peaks: 3 from the three phosphates of the ATP molecule, one from phosphocreatine (PCr) and one from inorganic phosphates (Pi). There are two additional peaks representing phosphomonoesters (PME) and phosphodiesters (PDE), which are sometimes observed in normal muscle.

The single inorganic phosphate peak is really a summed peak of HPO$_4^{2-}$ and H$_2$PO$_4^-$. The more acidic form of Pi resonates at one position in the spectrum and the basic form resonates at a different position, further away from the PCr peak. However, because the protonation/deprotonation reaction is extremely fast on an MRS timescale, these individual peaks are not separated. The resulting single peak moves in position between these two extremes, depending on the relative concentrations of the acidic and basic forms. The titration curve of the Pi is in the physiological pH range, thus its position reports on the pH environment. The intracellular water space is at least 5 times larger than the extracellular water space in normal skeletal muscle and the intracellular phosphate concentration is approximately 2 times larger than the extracellular phosphate concentration. Therefore, the vast majority of the Pi (>90%) is intracellular under normal circumstances. Since most of the intracellular water is cytoplasmic and since Pi in the mitochondria may be bound too tightly to be “seen” by MRS, the Pi observed by MRS is primarily cytoplasmic Pi. If the top of the Pi peak is used to determine the pH environment, this will always report on the intracellular component. Thus, the pH reported by the Pi peak will represent an average intracellular pH in the volume of muscle under the coil.

The concentration of Pi in the cytoplasm has been a matter of some debate since traditional methods for its measurement are liable to artefact resulting from phosphocreatine hydrolysis (during the delay for freezing of the tissue) and because of the presence of bound phosphate (not measured by MRS but included by some other analytical methods). MRS is the only way to measure Pi noninvasively in vivo. However, MRS measures only “free” mobile phosphate in solution in the cytoplasm. This component of Pi is important, however, because it is this metabolically active Pi that is involved in the control of energy metabolism. The relationship between extracellular Pi and intracellular Pi has been studied in patients with kidney failure who have variable levels of increased serum (and presumably extracellular) Pi [9,31]. It was found that high serum Pi is linearly correlated with high intracellular Pi. Therefore, at least one source of raised intracellular Pi is not directly related
to intracellular processes involved with energy metabolism.

During muscle work, PCr hydrolysis produces Pi on a mol for mol basis, but the increase in cytoplasmic Pi is slightly less than stoichiometric because of intracellular buffering of Pi. Pi is returned to normal concentrations after exercise by two important active mechanisms: mitochondrial uptake and ATP resynthesis. Pi and ADP must be transported into the mitochondria for the mitochondria to rephosphorylate ADP to make ATP. The transport of Pi into the mitochondria is mediated by a carrier that is specific for HPO$_4^{2-}$ and is pH dependent [12, 28]. This rate of transport is determined by two opposing factors. Low cytosolic pH increases the rate, but also decreases the proportion of HPO$_4^{2-}$. The optimum appears to be between 6.77 and 6.91. Transport out of the cell is too slow to contribute significantly to the recovery after exercise as shown in studies in vitro [24] and by the fact that the total signal from phosphate (sum of all peak areas) remains constant during such acute perturbations.

During recovery from exercise in which large quantities of Pi have been produced there is an undershoot in MRS-visible Pi ("Pi disappearance"). It has been speculated, but not proven, that some Pi may become “MRS invisible” (either as a result of binding to macromolecules or crystallization in mitochondria [11, 12]). However, this invisibility is still under debate as to its cause. Alternate suggestions have been that the undershoot is due to broadening of the Pi peak as a result of pH heterogeneity between fiber types [18] or that it is due to trapping of Pi in sugar phosphates generated by anaerobic glycolysis [8, 33].

Cytosolic phosphorylation potential (PP), defined by the equation below.

\[
PP = \frac{[ATP]}{[ADP] \times [Pi]}
\]

is proportional to the free energy of hydrolysis of ATP, and thus provides information about the amount of energy that mitochondria are able to "store" in ATP and the ability of cells to perform work. This ratio is believed to play a role in the control of mitochondrial respiration. ATP in the cytoplasm is in chemical equilibrium with PCr through the reaction catalyzed by creatine kinase (CK):

\[
PCr + ADP + H^+ \rightleftharpoons ATP + Cr
\]

Equation (2) can be expressed as the chemical equation below.

\[
[ADP] = \frac{[ATP] \times [Cr]}{[PCr] \times K \times [H]}
\]

which when substituted in equation (1) gives:

\[
PP = \frac{[PCr]}{[Pi]} \times K \times [H] \times \frac{1}{[Cr]}
\]

From equation 4, it can be seen that the cellular energy state is proportional to the ratio of [PCr]/[Pi], which can be measured by phosphorus MRS. Thus, [PCr]/[Pi] has been extensively used in metabolic studies as a measure of the energy state of muscle in vivo.

Considerable emphasis has been placed by us and others on the possibility of using the CK equilibrium constant and measurement of relative PCr/ATP concentrations and intracellular pH to estimate free ADP concentrations in muscle using equation [3]. This calculation depends on a number of reasonable assumptions [3, 4]. In contrast to the estimation of energy state from the ratio of PCr/Pi, the calculated cytosolic [ADP] does not involve the [Pi]. Several lines of evidence suggest that ADP is an important regulator of mitochondrial (oxidative) metabolism, and its levels can be used to assess the energy state of the cell independent of Pi. There is a debate about the control that intracellular Pi exerts on mitochondrial metabolism [7]. It is probably a major controller in unique conditions only, but may contribute to overall control, as proposed in the model of multiple controls [10].

**Altered muscle phosphate metabolism in CNS disorders**

**A. CNS disorders associated with primary mitochondrial dysfunction in muscle**

Primary mitochondrial disorders are defined as disorders in which the basic defect is in mitochondrial metabolism [13]. This is meant to distinguish them from disorders in which secondary mitochondrial dysfunction occurs, e.g. chronic ischemia or muscle damage from denervation, or simply deconditioning. These disorders result in muscle and brain dysfunction in recognizable combinations (encephalomyopathies). Any attempt to evaluated muscle phosphate metabolism, especially high energy phosphates, in CNS diseases must take into account the possible common denominator of primary mitochondrial abnormality in both organs. MRS can be used to detect mitochondrial dysfunction in these disorders based on alterations in the normal ratios of high to low energy phosphates at rest, or based on evidence for slow ATP resynthesis after exercise. ATP resynthesis rate after exercise can be inferred from the rate of recovery of PCr or free ADP [4].

Several series of patients with mitochondrial encephalomyopathies studied by muscle phosphorus MRS have been reported [1, 5, 17, 19, 29]. Despite the differences in protocols and patient selection, the major findings in these series are quite similar: phosphorus MRS of muscle is abnormal in more than 80% of patients with mitochondrial encephalomyopathies. We will describe our findings in a group of 32 patients with well-defined, biopsy-proven mitochondrial disorders studied at the Montreal Neurological Institute, as a prototype to the possible abnormalities observed by phosphorus MRS in other CNS diseases.

**Muscle at rest:** Of the 32 patients, 22 had abnormal ratios...