Investigational Methodologies for the Effects of Brain Maturation on Energy Transport

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Abstract. Intracellular kinetics of high energy phosphate (HEP) is of fundamental importance in cellular biochemical physiology. In mammalian brain, intracellular HEP transport from the production site (mitochondria) to the consumption site (plasma membrane) is dependent on passive diffusion of HEP through the cytosol. Diffusivity of a substrate in a solution correlates inversely to the viscosity of the solution. The maturational process of mammalian brain involves dramatic changes in the cytosolic amino acid profile. Since the viscosity of a solution is a function of the diffusion coefficients of solutes and their concentrations, changes in the cytosolic amino acid composition should result in significant alteration in cytosol viscosity and hence, HEP diffusivity. Such a system is especially suitable for mathematical modeling and correlative analysis by in vivo nuclear magnetic resonance (NMR) spectroscopy. This brief review is written to provide a fundamental background for investigational methodologies on developmental neurobiology of cellular energetics. (Keio J Med 41 (2): 64–67, June 1992)

Key words: adenosine triphosphate, phosphocreatine, molecular diffusion, NMR

Introduction

Intracellular transport of certain biologically significant substrates is purely dependent on their passive diffusion through the cytosol. One of the best examples is the diffusion of adenosine triphosphate (ATP). In mammalian brain, ATP is primarily produced in the mitochondria by oxidative phosphorylation and transported to the plasma membrane where most of the ATP is utilized to maintain properties essential for brain function, namely, membrane excitability.1–4 The cytosolic microenvironment is an essential parameter for defining cytosolic viscosity and, hence, diffusivity of passively diffusing substrates.5 The maturational process of brain in various mammalian species including rats and humans involves dramatic changes in the concentration of free amino acids in the cytosol. In fetal brain, taurine is high while N-acetyl-aspartate (NAA) is low. After birth, this relationship is rapidly reversed to the NAA rich cytosol of adult brain (taurine/NAA exchange).6–10 In principle, such marked changes in the composition of the cytosol result in significant changes in cytosolic viscosity and hence, diffusivity of ATP. Indeed, recent studies disclosed that intracellular high energy phosphate (HEP) transport in rat brain undergoes dramatic alteration after birth as part of the postnatal adaptational process.11 Facilitation of HEP transport in the higher energy consuming adult brain appears to be a main function of NAA. The purpose of this brief review is to provide the methodological background for investigations on the effects of brain maturation on intracellular HEP kinetics.

Theory

Diffusion of particles

Fick’s law for the particle flux density is given by

\[ \mathbf{J}_n = -D_n \nabla n \]

where \( D_n \) is the diffusion coefficient and \( n \) is the concentration of the particle.5,12

The equation of continuity, \( \partial n / \partial t + \nabla \cdot \mathbf{J}_n = 0 \), assures that the number of particles is conserved. Since \( \nabla \cdot \nabla = \nabla^2 \) (Laplacian), the diffusion function is then given by

\[ D_n \nabla^2 n = \frac{1}{\sqrt{4\pi D_t}} \exp\left(-\frac{x^2}{4D_t}\right) \]

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which is normalized to the area \( \int_{-\infty}^{\infty} \theta(x,0)dx = 1 \) is known to conform to the diffusion function and can be used for diffusional length evaluation.\(^{5,12-13}\)

The pulse spreads out with increasing time. After evaluating the Gaussian integral, the mean square value of \( x \) can be given by: \(<x^2(t)> = \frac{\int_{-\infty}^{\infty} x^2 \theta(x,t)dx}{\int_{-\infty}^{\infty} \theta(x,t)dx} = 2Dt\). The root mean square value is, therefore, \( x_{rms} = \sqrt{<x^2(t)>} = \sqrt{2Dt} \). This equation provides the distance for the particle to travel in one dimension (diffusion length) in a given time, \( t \). For biological substrate such as ATP, the time can be defined as the life span which is the inverse of the turnover rate (see Experimental Procedures section for further discussion).

**Diffusion of particles in solution**

Diffusivity of particles immersed in solution is inversely correlated to the viscosity of the solution, \( \eta \), \( D \propto \frac{1}{\eta} \). Viscosity is a function of its own diffusivity, \( D_\alpha \), and concentration, \( \rho \), and is given by, \( \eta = \rho D_\alpha \).

The Stokes-Einstein relation provides the diffusion coefficient of the particle the radius of which is \( R \),

\[
D = \frac{\tau}{6\pi\eta R},
\]

where \( \tau \) is the fundamental temperature (4.28 \times 10^{-14} \text{ ergs for biological tissue temperature of 37°C}).\(^{5}\)

**Model**

Each substrate diffusing freely in the cytosol contributes to the viscosity of the cytosol. Assuming that the density distribution of each substrate is uniform in the cytosol, the viscosity of cytosol, \( \eta_c \), can be expressed as: \( \eta_c = \sum \rho_i D_i f_i \) where \( f_i \) is the contribution factor of each substrate.

Among those substrates freely diffusing in the cytosol, only taurine and NAA show significant changes in their concentration during postnatal development. Therefore, the above equation can be reduced to: \( \eta_c = \text{const} + \rho_\text{taur} D_{\text{taur}} + \rho_\text{NAA} D_{\text{NAA}} \) where subscripts \( t \) and \( n \) represent taurine and NAA, respectively.

\( D_t, D_n, \rho_t, \) and \( \rho_n \) can be directly determined experimentally. Other parameters can be determined indirectly by measuring the diffusion coefficient of HEP.

**Experimental Procedures**

Actual experiments on HEP kinetics are almost entirely dependent on techniques of in vivo nuclear magnetic resonance (NMR) spectroscopy.\(^{14-16}\) A detailed discussion of currently available NMR techniques is not the intent of this small review. However, it cannot be overemphasized that it would have been virtually impossible to perform kinetic analysis of HEP in vivo without NMR. Here, two specific techniques indispensable for HEP kinetics, namely, diffusion spectroscopy and the saturation transfer experiment, will be discussed.

**Diffusion coefficient determination by diffusion spectroscopy**

The measurement of self-diffusion coefficients by NMR originally described by Hahn\(^{17}\) and Carr and Purcell,\(^{18}\) later modified by Stejskal and Tanner,\(^{19}\) is a sensitive technique for assessing the microenvironment of a solution with respect to molecular diffusion.

When there is no diffusion or gradient pulse, the echo signal amplitude of the nth echo, \( A_{(n)} \), can be given by:

\[
A_{(n)} = A_0 \exp \left( -\frac{\gamma^2 G^2 D \tau^2}{12} \right)
\]

where \( A_0 \) is signal intensity at time 0 and TE an \( T_2 \) are echo time and transverse relaxation time, respectively. Under a gradient pulse, \( G \) gauss/cm, and molecular diffusion with coefficient value, \( D \) mm\(^2\)/sec, the echo signals, \( A^* \), will be reduced by a factor, \( B \), as follows: \( A^* = A_{(n)} B \) where \( B = \exp \left( -\frac{\gamma^2 G^2 D \tau^2}{12} \right) \).

Using the Stejskal-Tanner sequence (Fig 1), \( B \) can be expressed as: \( B = \exp \left( -\gamma G D B_0 (\Delta - \delta/3) \right) \).

By varying either diffusion time (\( \Delta - \delta/3 \)) or gradient strength, the diffusion coefficient can be determined as a function of diffusion time from the slope of the plot for \( \ln(A_{(n)}/A_{(0)}) \) based on the equation: \( \ln(A_{(n)})/A_{(0)} = -\gamma^2 G^2 D \delta^2 (\Delta - \delta/3) \) where \( A_{(n)}/A_{(0)} \) is the ratio of echo signal amplitudes in the presence and absence of a gradient.

Considering various NMR parameters, the proton resonance for -CH\(_3\) (singlet) and -CH\(_2\) at C-2 (triplet) for NAA and taurine and \(^{31}\)P resonance for PCr (singlet) and \( \alpha\)-ATP (duplet) are most suitable for in vivo investigation. In order to minimize signal attenuation secondary to phase modulation due to scalar coupling for multiplets, the interval between the 90° and 180° pulse should be
fixed at 68 msec for proton and 25 msec for $^{31}$P. For in vivo proton studies where suppression of tissue water signals in necessary, the 90° and 180° pulse should be replaced by a high order Hore sequence, 1331 and 2662, respectively.\textsuperscript{20}

**HEP life span measurement by saturation transfer experiments**

The steady state saturation transfer (SSST) experiment represents one of the well established NMR magnetization transfer experiments widely utilized for rate constant determination of creatine phosphokinase (CPK).\textsuperscript{21 - 24} It has been known that SSST experiments provide an accurate forward rate constant (PCr $\rightarrow$ ATP) for the CPK reaction, although they tend to underestimate the reverse rate constant (ATP $\rightarrow$ PCr). Therefore, the SSST can be effectively utilized to assess the flux of CPK reaction under steady state conditions in vivo.

The first order rate constant for the forward reaction can be determined using the following equation:

$$K = \frac{1 - M_s/M_0}{T_{1\text{app}}},$$

where $K$ is the rate constant, $M_0$ is the magnetization (PCr) without saturation on the exchanging partner (γ-ATP), $M_s$ is the magnetization with saturation on the exchanging partner, and $T_{1\text{app}}$ is the apparent $T_1$ measured with saturation on the exchanging partner.

The intracellular level of PCr, [PCr], can be given by:

$$[\text{PCr}] = [\text{ATP}] \times \frac{H_{p/a}}{H_{p/a}},$$

where $H_{p/a}$ is the ratio of the Lorentzian corrected heights of the PCr and ATP resonances obtained from a fully relaxed $^{31}$P spectrum. The flux of the steady state CPK reaction is considered to be equal to the steady state turnover rate of ATP and, therefore, it is possible to calculate the life spans of PCr and ATP by dividing their respective concentrations by the flux of CPK: PCr life span = $\frac{[\text{PCr}]_{\text{flux}}}{[\text{PCr}] \times K}$, $\text{ATP life span} = \frac{[\text{ATP}]_{\text{flux}}}{[\text{ATP}] \times K} = \frac{1}{[\text{PCr}] \times K}$.

It is apparent, therefore, that regardless of the actual levels of PCr and ATP, life span of HEP can be calculated using only two parameters, namely, the forward rate constant, $K$, and steady state fully relaxed height ratio, $H_{p/a}$.

**Diffusion length calculation**

Using the life span value obtained by SSST experiments and appropriate diffusion coefficients, the diffusion length, the averaged distance HEPs diffuse prior to their consumption, can be calculated by the equation:

$$\text{Diffusion length} = \sqrt{2D\tau} \text{ where } \tau \text{ is the life span (see Theory section for further discussion).}$$

**Conclusion**

The mechanisms and kinetics of intracellular energy transport remain to be a fundamental topic in biochemical cellular physiology. Investigation on the effects of brain maturation on HEP kinetics is of critical importance for developmental neurobiology. The introduction of in vivo NMR spectroscopy has opened a new era of biochemical/biophysical investigation, especially those for substrates vulnerable to fixation artifact such as HEP. The capability of performing chemical/physiological analysis non-invasively in live animals by NMR is essential for analysis of HEP kinetics. The methodologies presented here can provide hitherto unobtainable parameters for brain HEP kinetics critical for investigations on brain maturation.

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**References**

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