Reassessment of the apparent equilibrium constant of Creatine Kinase Reaction for accurate in vivo assessment of [ADP] by 31P MRS in the human brain and skeletal muscle.

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Introduction

In vivo assessment of [ADP] in the human brain and skeletal muscle by 31P-MRS is essential to the knowledge of the functionality of mitochondrial respiration, hence of the degree of "health" of the tissue (1, 2). However, [ADP] cannot be directly measured in vivo by 31P-MRS in whole body magnets due to both its low concentration in the living tissues and the lack of sufficient resolution offered by 1.5 T magnets. As a consequence, [ADP] is usually calculated from the creatine kinase reaction. Hence, a precise knowledge of the apparent equilibrium constant of creatine kinase reaction (KCK-app) is essential for accurate in vivo quantification of [ADP]. Creatine Kinase catalyzes the magnesium-dependent reversible transphosphorilation between phosphocreatine (PCr) and ATP according to the reaction:

$$\sum PCr + \sum ADP = \sum ATP + Cr$$

where $\sum$ denotes the sum of all the ionic, acidic and metal-complexed forms. It is well known that the actual substrates of creatine kinase are the magnesium-complexed forms, but the determination of the equilibrium constant of the reaction requires the measurement of the concentration of the free ionic forms only. Since direct measurement of free ionic forms only is not feasible, it is convenient to use the apparent equilibrium constant (KCK-app) which takes into account the sum of all forms. KCK-app is very useful operatively, but has the drawback to be dependent on H+ and on metal ion concentrations. As a consequence, KCK-app needs to be defined as a function of the concentration of all the Lewis acid to be taken into account, precisely: Mg2+, H+, K+, Na+. To date, the commonly used value of KCK-app assumes free magnesium ion concentration of 1 mM to calculate [ADP] (3). However, it has been reported that in both human brain and skeletal muscle the concentration of free Mg2+ is far below 1 mM, and that in the human skeletal muscle [Mg2+] changes during exercise and recovery from exercise (4,5). It derives that in vivo assessment of [ADP] in the human brain and skeletal muscle by 31P MRS suffers from the bias of not taking into account values of [Mg2+] different from 1 mM (6). The only reported values of the KCK-app have been determined by Veech et al.(3), by using a model that takes into account only some of the magnesium complexes and of acidic forms of ATP, ADP and PCr. However, in in vivo systems K+ and Na+ ions participate to equilibria with ATP, ADP, and PCr, their intracellular concentration being sufficiently high. We present here a new model that allows an accurate quantification of [ADP] in living tissues as assessed by 31P-MRS. Our model takes into account: a) more magnesium complexes and acidic forms of ATP and ADP than the Veech model, b) the complexes formed by ATP, ADP and PCr with Na+ and K+, and makes use of a set of equilibrium constants critically revised and/or re-determined by potentiometric titration (4).

Methods

The equilibrium concentrations of all species, including free [Mg2+], were calculated by the computer program HYSS (7) using a set of equilibrium constants at 37°C and I = 0.25 M. The equilibrium constant of HATP3-, H2ATP2-, MgATP2- e MgHATP- were measured by potentiometric titration at 25° C and corrected for temperature by Van't Hoff equation (4). All others equilibrium constant were taken from the literature (8) and corrected both for temperature by Van't Hoff equation and ionic strength by Davies equation. KCK-app was obtained in the range of pMg 1-8 by adding the concentration of all free ionic, acidic, Mg-,K-,Na-complex forms of ATP,ADP and PCr calculated by HYSS at pH=7, [K+]=0.15 M, [Na+]=0.05 M. Then Kobs was defined as: Kobs = KCK-app x [H+] and calculated at pH=7.

Results

The chemical model used includes three ligands (ATP4-, ADP3-, and PCr2-) and four Lewis acids (Na+, K+, Mg2+ and H+). The cumulative constant of all equilibria considered in our chemical model are reported in the Table. Figure 1 reports four different patterns of Kobs plotted as a function of pMg and obtained according to the different approach and/or chemical models used. The pattern A1 is obtained by using the Veech model (3) which takes into account only the first association constant of ATP, ADP and PCr with Mg2+ and H+. The pattern B1 is obtained as A1 using a set of equilibrium constant revised and corrected. The pattern B2 is the result of our model which also takes into account the binding of ATP, ADP and PCr to K+ and Na+. B3 pattern shows the behavior of our model when the complex Mg2ATP is neglected.

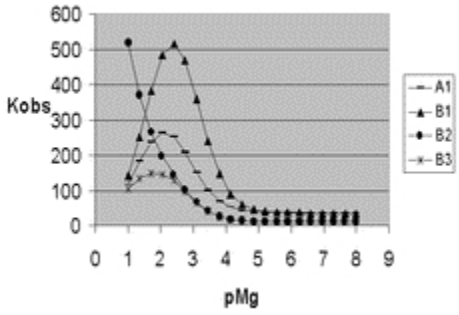

Figure 1

Discussion

These results show that, by using our new chemical model the actual cytosolic free [ADP] calculated from the creatine kinase equilibrium at physiologic concentration of free Mg2+ results twofold higher than previously assessed (9,10).

References

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Cumulative formation constants of the selected species

Species	Log K
HATP3-	6.79
H2ATP2-	10.63
NaATP3-	1.11
KATP3-	0.98
MgATP2-	4.60
MgHATP-	8.93
MgH2ATP	11.93
Mg2ATP	6.21
HADP2-	6.44
NaADP2-	0.97
KADP2-	0.85
MgADP-	3.22
MgHADP	7.85
MgPCr	1.60
HPCr-	4.46
KPCr-	1.30