Combination of ^{18}FDG-PET, ^{13}C MRS and ^{31}P MRS provides a highly consistent picture of brain energy metabolism, from glucose breakdown up to ATP synthesis

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Introduction

The attempt to measure brain energy metabolism *in vivo* has been driving considerable technological developments in medical imaging over the last decades. In the late 70[ies], positron emission tomography (PET) made it possible to measure the cerebral metabolic rate of glucose (CMRglc), namely the glycolytic step of ATP production [1]. About 15 years later, ^{13}C MRS yielded the first measurement of the cerebral TCA cycle rate V_{TCA}, i.e. the oxidative step of ATP production [2, 3]. In the same years, ^{31}P MRS led to the cerebral rate of ATP synthesis V_{ATP} [4, 5]. However some controversy still persists on the proper way to quantify V_{TCA} [3, 6], and only one measurement of cerebral V_{ATP} has been reported for rodents [4] and for humans [5]. Under normal physiological conditions, glucose breakdown (through glycolysis and TCA cycle) is stoechiometry coupled to ATP synthesis, so that simultaneous measurement of CMRglc, V_{TCA} and V_{ATP} would allow to cross-validate the 3 techniques.
In this context our purpose has been to measure CMRglc, V_{TCA} and V_{ATP} in the same macaque monkeys under identical physiological conditions. A cross comparison of these measurements in terms of energy metabolism is carried out, and the interest of this global approach is discussed.

Materials & Methods

The study was conducted on 2 healthy monkeys (*macaca fascicularis*; body weight ~8 kg). For PET and NMR sessions, animals were anesthetized using i.v. infusion of propofol (~200 µg/kg/min), intubated, ventilated and placed in the Sphinx position using a stereotaxic frame. PET was performed on an ECAT EXACT HR+ tomograph (Siemens-CTI, Knoxville, TN, USA). MR experiments were performed on a whole-body 3T system (Bruker, Ettlingen, Germany) equipped with a surface coil placed on top of the head (double-tunable ^{1}H-^{31}P, Ø~4.5cm). Both V_{TCA} and V_{ATP} sessions (performed with this same coil) started with the acquisition of gradient echo scout images in order to position the same 2×2×2cm^3 voxel of interest (VOI) in the upper brain. Then the voxel was shimmed using FASMAP.
A total of 23 sessions was conducted on the 2 monkeys: 14 NMR sessions for V_{ATP}, 5 NMR sessions for V_{TCA} and 4 PET sessions for CMRglc.
CMRglc measurement by ^{18}F PET Emission scans (4.5mm isotropic resolution) were collected during 60 minutes, following an i.v. bolus injection of ^{18}F-FDG (~2,5mCi). In order to correct time activity curves for [^{18}F] decay from the time of injection, arterial blood samples were withdrawn during the acquisition. Attenuation correction was performed [7]. Multi-modal superimposition of reconstructed ^{18}F-FDG images with gradient echo scout MRI was performed in order to localize the 2×2×2cm^3 VOI on the ^{18}F-FDG images. The time-activity curve was extracted from this voxel and CMRglc was calculated by a kinetic analysis based on a 2-tissue compartmental model [7].

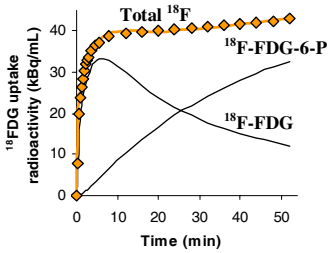

Fig. 1. ^{18}FDG PET time-activity curve and best fit by the 2-compartment model

V_{TCA} measurement by indirect ^{13}C NMR A baseline ^{1}H STEAM spectrum was acquired within the VOI (TE/TM/TR=21/110/2500, 256 transients). Then ^{1}H STEAM spectra were collected during a 2-hr i.v. infusion of [U-$^{13}C_6$] glucose. Blood samples were collected to measure glucose ^{13}C fractional enrichment (FE) during the infusion. Measurement of glutamate ^{13}C FE from the ^{1}H spectra was based on a previously described method [8]: basically, the subtraction of ^{1}H spectra acquired during the ^{13}C infusion from the baseline ^{1}H spectrum results in difference spectra where only labeled metabolites remain. V_{TCA} was derived from glutamate FE using a conventional mathematical model [8].

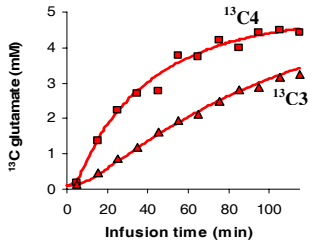

Fig. 2. Average glutamate ^{13}C4 and ^{13}C3 time-courses measured by NMR and best fits

V_{ATP} measurement by ^{31}P saturation transfer experiment ^{31}P spectra were collected from the VOI using an OVS-localized sequence, preceded with a saturation pulse (length t_{sat}) as previously described [9]. The saturation frequency was first set to γ-ATP frequency and spectra were collected for 4 different values of t_{sat} (0.5; 1.0; 1.5 and 2s) using a 2.95s TR and 512 transients for each t_{sat}. Symmetrical saturation relative to Pi was performed as a control in order to correct for RF bleed over effect on Pi. ^{31}P spectra were quantified using a basis set of thirteen metabolites implemented for AMARES in jMRUI [10, 11]. Pi attenuation as a function of t_{sat} was fitted in order to derive the unidirectional rate of ATP synthesis k_f. Then V_{ATP} was calculated using the equation: $V_{ATP}=k_f\times[Pi]$ [5].

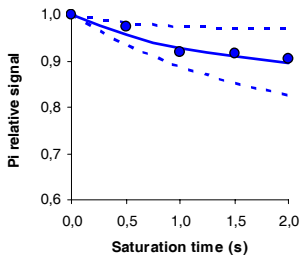

Fig. 3. Average Pi attenuation (♦) vs. saturation time t_{sat}, best fit (-) and lower and upper limits (dotted lines)

Results

Kinetic analysis of ^{18}F-FDG uptake yielded CMRglc=0.28±0.04µmol.g^{-1}.min^{-1} (Fig. 1). Figure 2 represents the time-courses of glutamate C3 and C4 FE. The best fit to these data leaded to V_{TCA}=0.64±0.02µmol.g^{-1}.min^{-1}. The best fit to Pi attenuation (Fig. 3) yielded k_f=0.11±0.04s^{-1} and V_{ATP}=7.9±2.9µmol.g^{-1}.min^{-1}.

Discussion

Taken independantly, the 3 fluxes are in good agreement with literature values of brain energy metabolism [5, 7]. More interestingly, they appear highly consistent with each other. Glucose being the main cerebral fuel under normal physiological conditions, a V_{TCA}/CMRglc ratio of 2 is expected since glycolysis produces 2 pyruvates per glucose [12]. The experimental ratio of 2.3 is very close to this prediction. In addition, optimized ATP synthesis associated with (i) mitochondrial production of NADH and $FADH_2$ (32 ATP/glucose) and (ii) direct ATP production from ADP (2 ATP/glucose along the glycolysis and 2 ATP/glucose along the TCA cycle) theoretically leads to a V_{ATP}/CMRglc ratio of 36. Our experimental ratio of 28 is slightly lower, possibly due to partial permeability of mitochondrial membrane to ^{1}H, or to the insensitivity of ^{31}P measurement to glycolytic ATP synthesis [5].
This study presents the first measurement of CMRglc,V_{TCA} and V_{ATP} in the same animals under identical physiological conditions, providing an integrated picture of energy metabolism which cross-validates the 3 corresponding quantitative techniques. Moreover, the ability to measure the 3 fluxes should prove usefull for studying energy deficit associated with pathological conditions like neurodegeneration.

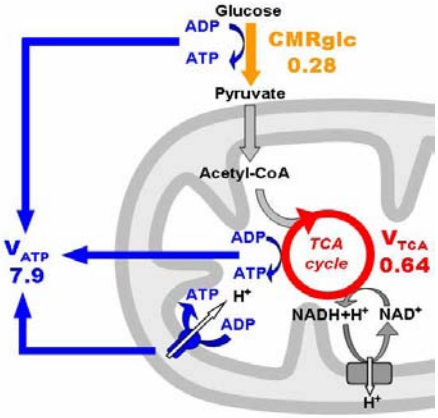

Fig 4. CMRglc, V_{TCA} and V_{ATP} measured in the monkey brain (in µmol.g^{-1}.min^{-1})

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