NMR Measurement of V_{TCA} Correlates with Enzymatic Activity along the TCA Cycle as measured by Histochemistry in the Primate Brain

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Introduction

During the past years, MR spectroscopy (MRS) has demonstrated its ability to explore brain metabolism based on the measurement of ^{13}C labeling of TCA cycle intermediates or metabolites such as glutamate following injection of ^{13}C-enriched substrates [1]. Quantitation of the TCA cycle flux (V_{TCA}) remains somewhat controversial, making it necessary to validate V_{TCA} measurements by comparison with an alternate measure of metabolic activity. Although this approach has been used to study actual brain disease or animal models [2-4], the extent to which NMR-measured V_{TCA} reflects enzymatic activity along the TCA cycle remains to be explored. Recently, we have reported a cross-validation of V_{TCA} measurements by comparing with the measurements of the glycolytic flux derived from ^{18}F-FDG positron emission tomography in healthy monkeys [5]. In this context, our objective has been to evaluate the ability of MRS to detect changes in enzymatic activity along the TCA cycle. To do so, we have induced a stable mitochondrial impairment by the means of chronic inhibition of succinate dehydrogenase (SDH) by the toxin 3-nitropropionic acid (3NP) [6]. For validation, we have compared, for one of the 3NP-treated monkey, the NMR-measured decrease in V_{TCA} to the SDH inhibition measured *post-mortem* with *in situ* histochemistry at the end of the 3NP treatment.

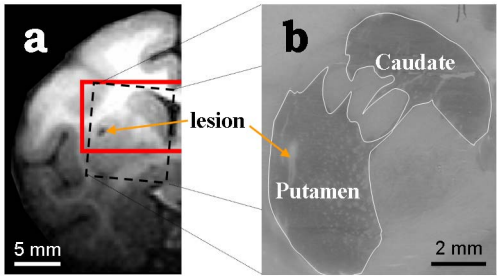

Fig. 1. A. T_1 MR image showing half of the spectroscopic voxel of interest centered on the striatum (in red, cal. bar: 5mm) and B. Corresponding *in situ* SDH histochemistry brain section from the same monkey (#1) (cal. bar: 2mm).

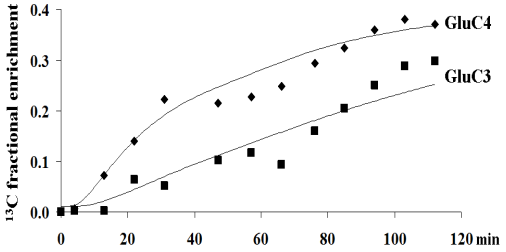

Fig. 2. Typical [4-^{13}C]glutamate and [3-^{13}C]glutamate labeling time-courses with best fits by a single compartment metabolic model.

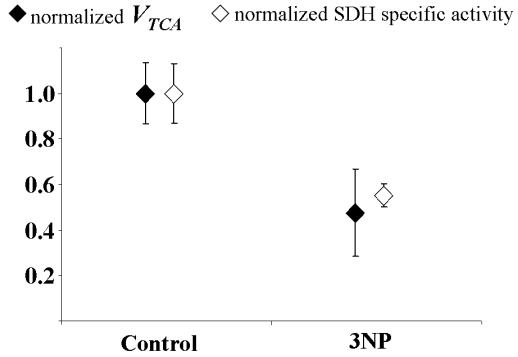

Fig. 3. Normalized TCA cycle flux (V_{TCA}) measured in the striatum before (Control, for all 3 monkeys) and during 3NP treatment (3NP, for monkey #1) and normalized SDH specific activities measured in the caudate and putamen for a group of 3 control monkeys (Control) compared to that of the sacrificed 3NP-treated monkey (#1). Means ± SD.

Materials and Methods

Animals and 3NP treatment. Three macaque monkeys (*macaca fascicularis*, 7–8 kg) were studied before and during 3NP treatment (Sigma Aldrich Co., St Louis, MO, USA) for 30 weeks. The neurotoxic treatment consisted of two daily i.m. injections with an initial dose of 10 mg/kg/day progressively increased at weekly intervals to 30 mg/kg/day as previously described [6]. Regularly (~ once every 5 weeks), MRS experiments were performed under propofol anaesthesia (i.v. infusion, 200 µg/kg/min), the animal being ventilated. After completion of the MRS study (at the 37th week of 3NP intoxication), one of the three monkey (#1) was sacrificed by an overdose of pentobarbital (100 mg/kg; Sanofi, Marnes-la-Coquette, France) and transcardially perfused with cold saline. Then the brain was removed immediately, hemisected by a midline sagittal cut, slabbed on a monkey brain slicer and rapidly frozen in dry ice for SDH histochemistry.

Data acquisition. NMR Experiments were performed on a 3 Tesla whole-body system (Bruker, Ettlingen, Germany) equipped with a ^1H surface coil. T_1 images were acquired for positioning of the 30×10×13mm^3 voxel in the striatum (Fig.1A). After shimming down to 7 Hz, a baseline ^1H PRESS spectrum was acquired (TE/TR = 8/2500ms, 432 scans) then spectra were collected during a 2-hour i.v. infusion of [U-$^{13}C_6$]glucose (9-min time resolution). Blood samples were collected for glucose ^{13}C enrichment measurement.

TCA cycle flux. ^{13}C incorporation into brain glutamate C4 and C3 was detected on the ^1H PRESS spectra as described elsewhere [7]. Time-courses of glutamate C4 and C3 fractional enrichment (Fig. 2) were fitted by a single compartment metabolic model describing ^{13}C incorporation from glucose to brain glutamate [8], leading to V_{TCA}.

In situ SDH specific activity. SDH specific activity was evaluated *in situ*, using a histochemical procedure [9] and a previously described quantification method [10]. For 3NP values, SDH specific activity values were averaged over 2 slices while for control values, values were averaged over 3 slices each extracted from a different monkey. 3NP-treatment induced SDH inhibition was estimated in the putamen and caudate (Fig 1B) by comparing SDH specific activities in the monkey #1 to whose measured in 3 control brain monkeys.

Results

The V_{TCA} values measured before and over the 30-week intoxication protocol are exhibiting an immediate, prolonged and highly significant 44% decrease upon intoxication (from 0.58 ± 0.08 to 0.33 ± 0.10 µmol/g/min, mean±SD; p<0.00001, ANOVA). Similarly, a general decrease in SDH activity was observed in the putamen and caudate of the monkey #1 treated with 3NP for 37 weeks, as compared with the control group (Fig.2). Measurements of SDH specific activity confirmed a highly significant SDH inhibition in the putamen (-48%, p=0.035) and the caudate nuclei (-42%, p=0.016). No significant changes in neuron or glia density were observed using NeuN and GFAP immunolabeling (data not shown). Thus, it seems that none of the metabolic alterations observed can be due to significant neuronal loss or astrogliosis.

Conclusion

In this study, we induced a steady mitochondrial impairment by means of the inhibition of the SDH using chronic 3NP injection. This inhibition has led to a major decrease of the oxidative energy metabolism as measured with ^1H-{^{13}C} MRS *in vivo* (mean -44%, -52% for the sacrificed monkey). *Post-mortem* measurement of SDH inhibition using *in situ* histochemistry has led to a remarkably similar estimation (putamen: 48%; caudate: 42%). This comparison constitutes a strong argument to support the validity of *in vivo* MRS measurement of V_{TCA}. Besides, this study demonstrates that 3NP treatment could be an interesting model to study the relationship between SDH activity and the actual oxidative synthesis of energy. However, the sacrifice of primates being difficult on an ethical and practical point of view, the study should rather be realized on rodents.

References

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