Isoflurane strongly affects the diffusion of intracellular metabolites in the brain, revealing the contribution of subcellular restriction to metabolite ADC

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Introduction

Diffusion-weighted (DW) NMR spectroscopy is a unique tool to explore brain cells *in vivo* under normal or pathological conditions (for review see [1]). Halogenated volatile anesthetics such as isoflurane or halothane are commonly used for DW-NMR spectroscopy animal studies of the brain. However, it has been proven *in vitro* that volatile anesthetics induce changes in lipid bilayers fluidity, viscosity and permeability [2,3]. Since brain metabolites hardly cross lipid bilayers under normal conditions, perturbation of membrane properties may potentially affect metabolite diffusion. A potential effect of isoflurane on diffusion may reveal critical for comparative studies. It could also give an insight into the restriction effects which govern metabolite diffusion *in vivo*. In this context our primary objective has been to test the hypothesis of metabolite diffusion changing with isoflurane dose. ADC was measured in the brain of monkeys anaesthetized under 2 different doses of isoflurane using diffusion tensor spectroscopy (DTS). Analysis of isoflurane-induced diffusion changes for 5 different metabolites argues in favor of isoflurane increasing the permeability of subcellular organelles to metabolites.

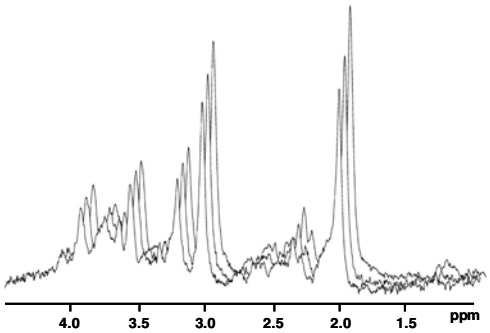

Fig. 1: Stack-plot of DW-spectra in the XZ direction.

Materials and Methods

Animal preparation 5 experiments were performed on 2 healthy macaque monkeys (*macaca fascicularis*). Primary anesthesia was induced by a small i.m. ketamine-xylazine injection. Animals were intubated and ventilated. They were held in the sphinx position and the head was positioned in a stereotaxic frame. Isoflurane dose was first set to 1% and DTS was performed (~1h acquisition). Isoflurane dose was then set to 2% and acquisition was stopped for 30-45min in order to allow for physiological parameters stabilization (decreased blood pressure). Another complete DTS acquisition was then performed.

NMR acquisition and processing Experiments were performed on a whole-body 3T system (gradient coil reaching 44mT/m). A surface coil was used for optimized detection of a cubic 5.8mL voxel in the fronto-parietal lobe. The DW-sequence consisted in a modified STEAM sequence with TE/TM=21/110ms [4]. Following a first acquisition at b=0, spectra were acquired at b~1000 and 2000s/mm² in directions XZ, -XZ, YZ, Y-Z, XY, -XY for determination of the diffusion tensor. Individual scan phasing was performed. A metabolite-nulling experiment was performed in order to subtract macromolecules from all DW-spectra. Spectra were then analyzed with LCModel [5]. The diffusion tensor **D** was derived for NAA, Cr, Cho, Ins and glutamate. The trace apparent diffusion coefficient (ADC) was finally derived.

Results and Discussion

Effect of isoflurane on metabolite diffusion A stack-plot of DW-signal attenuation (along the XZ direction) is shown on Fig. 1. ADC change was evaluated for each set of 2 successive experiments by a Student paired t-test, demonstrating a significant ADC increase when increasing isoflurane dose from 1 to 2% (Table 1).

Isoflurane effect on membrane permeability The increase in metabolite ADC might be ascribed to increased membrane permeability upon isoflurane [2,3]. This interpretation is supported by the fact that water diffusion is less affected by isoflurane (Table 1): indeed membranes are largely permeable to water molecules. However the release of metabolites from the intracellular to the extracellular space is very unlikely, as supported by the study by Pfeuffer *et al.* [6] which did not report any extracellular component in the rat brain under 2% isoflurane, for the 5 metabolites detected here. This suggests that isoflurane has little effect on plasma membranes permeability *in vivo*. In this context, increased metabolite ADC might be explained by facilitated exchange between subcellular spaces, resulting in a homogenized, less hindered intracellular diffusion space. Arguments favoring this interpretation can be inferred from the comparison of ADC changes measured for the 5 metabolites.

	ADC 1%	ADC 2%	ADC increase
NAA	0.136±0.006	0.155±0.021	14±13% (p<0.05)
Cr	0.106±0.016	0.135±0.022	27±7% (p<0.001)
Glu	0.223±0.026	0.263±0.029	20±18% (p<0.03)
Cho	0.094±0.010	0.144±0.022	53±17% (p<0.002)
Ins	0.192±0.019	0.217±0.030	13±8% (p<0.02)
Water	0.680±0.016	0.697±0.011	3±0% (p<0.05)

Table 1: ADC (µm²/ms) under 1% and 2% isoflurane, and ADC variation (N=5, except for water N=3).

Negative correlation between ADC and ADC change For metabolites experiencing significant restriction, it can be shown that ADC is relatively insensitive to free diffusion and rather dominated by the geometry of the diffusion space (as derived from theoretical models [7]). Thus ADC differences between metabolites primarily reflect differences in restriction. In this context increasing organelle permeability should have a stronger effect on heavily restricted metabolites. In other words, metabolites having a low ADC such as Cr or Cho (Table 1) should experience a stronger ADC increase under 2% isoflurane as compared with metabolites having a high ADC (Glu or Ins). As shown on Fig. 2, this prediction is verified by our data exhibiting a negative correlation between ADC and ADC increase upon isoflurane, suggesting that subcellular restriction is partly released by isoflurane.

Metabolite ADCs converge under the action of isoflurane Another way to test the hypothesis of isoflurane homogenizing diffusion within the intracellular space is to calculate ADC dispersion among metabolites: D=s.d.(ADC)/mean(ADC). Performing the calculation for NAA, Cr and Cho allows to compare all DW-MRS studies reported in the literature. For studies performed with halogenated volatile anesthetics, ADC dispersion (D=6±3%, 7 studies [6,8-13]) is about twice as low as for studies performed with other (or without) anesthetics (D=13±4%, 4 studies [4,14-16]). In the present report, D=7% under high isoflurane dose as compared with D=19% under lower isoflurane dose. Altogether these elements demonstrate that metabolite ADCs converge under the action of isoflurane, arguing in favor of isoflurane homogenizing the intracellular diffusion space. The interpretation of isoflurane affecting primarily organelle membranes is consistent with the specific composition of organelle membranes like the low cholesterol content as compared to the plasma membrane (cholesterol is known to govern the fluidity of biological membranes [17,18]).

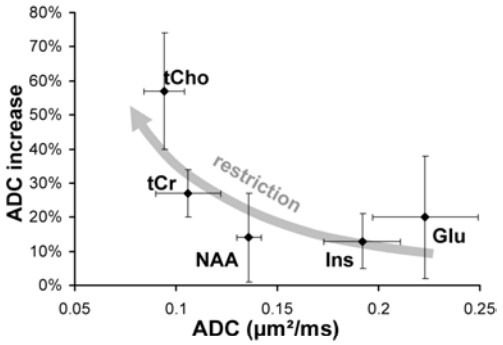

Fig. 2: Negative correlation between ADC and ADC increase.

Conclusion

In the present study it was shown that the ADC of intracellular metabolites is significantly increased at high isoflurane dose in the monkey brain. Analysis of ADC increases experienced by the 5 detected metabolites shows that ADC changes are negatively correlated to ADC values, and that metabolite ADCs converge under the action of isoflurane. Taking into account the reported effects of halogenated volatile anesthetics on biological membranes, these findings are interpreted as a partial release of subcellular restriction at high isoflurane dose. This work argues in favour of restriction by subcellular membranes being of critical importance for metabolite diffusion at the time-scale of *in vivo* DW-NMR spectroscopy (~100ms). The attenuation of subcellular compartmentation by volatile anesthetics may have strong physiological implications. For example, an increase in the permeability of mitochondrial membrane under the action of isoflurane could explain why oxidative metabolism is inhibited by isoflurane.

[1] Nicolay et al., NMR Biomed 14, p94 (2001) ; [2] Trudell et al., Biochim Biophys Acta 291, p321 (1973) ; [3] Qin et al., Biochemistry 34, p5536 (1995) ; [4] Valette et al., NMR Biomed, in press ; [5] Provencher, MRM 30, p672 (1993) ; [6] Pfeuffer et al., JCBFM 20, p736 (2000) ; [7] Tanner et al., J Chem Phys 49, p1768 (1968) ; [8] Merboldt et al., MRM 29, p125 (1993) ; [9] Wick et al., Stroke 26, p1930 (1995) ; [10] van der Toorn et al., MRM 36, p914 (1996) ; [11] Dijkhuizen et al., JCBFM 19, p341 (1999) ; [12] Dreher et al., MRM 45, p383 (2001) ; [13] de Graaf et al., MRM 45, p741 (2001) ; [14] Posse et al., Radiology 188, p719 (1993) ; [15] Harada et al., NMR Biomed 15, p69 (2002) ; [16] Ellegood et al., MRM 53, p1025 (2005) ; [17] Shechter, Biochimie et Biophys. des Membranes (1990) ; [18] Gaus et al., PNAS 100, p15554 (2003).