Combining Tissue Extract NMR, HRMAS and in-vivo MRS for Metabolic Studies

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Introduction

A range of NMR and MRS techniques can be used to study small metabolite brain metabolism. Each with their own advantages and disadvantages. In-vivo ^{1}H spectroscopy provides the most biologically relevant data, but at the expense of spectral sensitivity, limiting the data to ~15 major metabolites. Performing a polar extraction technique on excised brain tissue, produces NMR samples in which dozens of metabolites can be readily quantified, but at the expense of sample preparation. HR-MAS represents a compromise, using MAS to study excised tissue directly. By combining data from these techniques, along with a number of other NMR methods such as dissolution DNP, work is ongoing to create a toolbox that allows for a more complete understanding of brain metabolism than can be achieved with any single technique. Due to the range of equipment available, AMRIS provides a unique opportunity to conduct this research. Furthermore, contrasting these different techniques suggests a number of technique development opportunities to further improve our understanding of brain metabolism.

Experimental

Figure 1 provides an overview of the various MRS and NMR techniques performed in-vivo in living rats on the 11.1T imaging system, and ex-vivo on brain tissue on a 600 MHz Bruker spectrometer (both using HR-MAS and methanol/ chloroform extraction with solution state NMR)[1].

Results and Discussion

MATLAB fitting routines that fit a preselected list of metabolite peaks to the NMR data have been developed. The developed algorithms also allows for the line shape to be predicted from the given dataset, which produces an improved fit, particularly in HR-MAS samples where shimming can be problematic. Using a universal approach to peak fitting across the different NMR spectra acquired, allows for more robust comparisons between the data and a reduction in variability due to differences between peak fitting approaches. The cross technique comparisons have led to a number of interesting observations that warrant further research. There are a number of significant differences seen in relative metabolite concentrations between the HR-MAS, polar extract and in-vivo MRS. Some, such as the large increase in lactate in the deceased tissue are easy to explain; it is due to the anaerobic respiration during the hypoxic conditions present in the brain as the animal is sacrificed. Succinate is clearly seen in polar extract samples, while it is virtually undetectable in HR-MAS samples from the same animal. This apparent difference in concentration is still under investigation, but currently is believed to be due to the location of the succinate within sample. If the succinate's molecular motion is significantly restricted, due to is specific location in the cells within the brain tissue, then the shortened T_2* would greatly decrease the apparent signal intensity. The extensive tissue processing during the polar extraction frees the succinate, allowing it to be accurately measured.

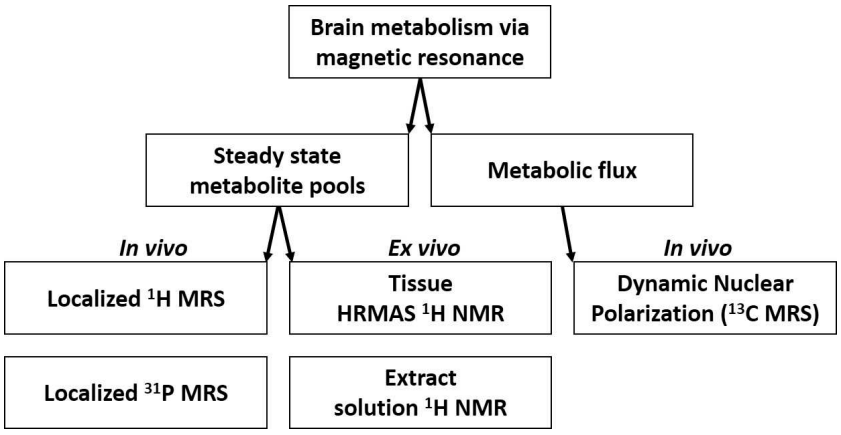

Fig.1 *Overview of Brain Metabolism MRS 'toolbox' and the range of potential magnetic resonance techniques which can be employed.*

Conclusions

Combining different MRS techniques allows for an improved understanding of brain metabolism. Comparisons between the spectra seen in the various techniques, allows for a more complete understanding of these metabolic processes, and can reveal interesting features that would otherwise be overlooked. Furthermore, the ongoing work suggests a number of potential future avenues of development, taking techniques from traditional ss-NMR and applying them to in-vivo MRS.

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References

[1] Downes, H., *et. al.*, ChemPhysChem, DOI:10.1002/cphc.201800917 (2018)