Hypoxia-induced metabolic alterations in hippocampal slices are fully reversible by reperfusion: a $^{13}$C NMR isotopomer analysis

J. N. Duarte, R. A. Cunha, R. A. Carvalho; Biochemistry, Center for Neurosciences and Cellular Biology, Coimbra, PORTUGAL, Department of Biochemistry, Faculty of Sciences and Technology, University of Coimbra, Coimbra, PORTUGAL.

Introduction: Hypoxia rapidly compromises neuronal function in brain tissue: cellular ATP levels decrease, excitatory amino acids are released and cellular ion gradients collapse, with resulting membrane depolarization (Trends Neurosci 17, 251). It has been shown that severe and mild hypoxia have some effects on labeling and on pool sizes of TCA-cycle-related amino acids, and induce large increases in the labeling of lactate and alanine as well as an increase in the pool size of lactate (Biochem J 291, 915). $^{13}$C NMR isotopomer analysis has proven to be a powerful tool for monitoring changes in intermediary metabolism. In this study we evaluated the effects of hypoxia in intermediary metabolism using hippocampal slices superfused with the tracers [2-$^{13}$C]acetate and [U-$^{13}$C]glucose.

Methods: Hippocampal slice superfusion: male Wistar rats (6 weeks old) were anesthetized with halothane and decapitated. Hippocampal slices were prepared with 400µm thickness. After a 45min resting in a Krebs solution gased with 95%O$_2$/5%CO$_2$, at room temperature, the slices were superfused (3mL/min) with the same solution during 60min, at 37°C, to stabilize. The slices were then superfused during 90min with this medium plus 50µM 4-amino-pyridine (4-AP), to allow slice stimulation, either in normoxic or hypoxic conditions (perfusion bubbled with 95%N$_2$/5%CO$_2$). Finally, slices were superfused during 3hours in normoxic conditions with a medium containing 4-AP, 5.5 mM [U-$^{13}$C]glucose and 2 mM [2-$^{13}$C]acetate. $^{13}$C NMR: Perchloric acid extracts of hippocampal slices were lyophilized, dissolved in 600µL D$_2$O and pH adjusted to 7.0. Proton decoupled $^{13}$C NMR spectra were acquired at 125.7 MHz on a Varian Unity-500 spectrometer using a 5mm broadband NMR probe, a 45º observe pulse, and a 3s interpulse delay. NMR spectra were processed using the PC-based software NUTS$^TM$.

Results/Discussion: The figure shows the expansions of glutamate C4 and GABA C2 resonances from $^{13}$C NMR spectra of extracts from superfused hippocampal slices.

We did not find any significant differences in the multiplet components of glutamate C4 and GABA C2 between hypoxia plus reperfusion and normoxia. Thus, we conclude that the possible metabolic modifications that occur in hippocampal slices during hypoxia are reverted during the reperfusion period, as occurs with the hypoxia-induced decrease in synaptic transmission (J Neurosci 21, 8564). When comparing the multiplet components between glutamate C4 and GABA C2 resonances we conclude that multiply-enriched isotopomers are more abundant in GABA, which is consistent with a higher GABAergic relative to glutamatergic activity in the hippocampus.