Measurement of Metabolic Rates in Rat Olfactory Bulb by 1H and 1H-[13C] NMR In Vivo

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INTRODUCTION: MRI/S at high magnetic fields has generated new insights into brain structure, function, and metabolism. Although extensive data related to high-resolution MRS of rodent cortex are available, MRS studies on the olfactory bulb (OB) are limited due to the small size of this structure and its proximity to bone and nasal cavities, which distorts field homogeneity leading to losses in sensitivity and resolution. In the present study, we measured rates of glutamatergic and GABAergic neurotransmission, in addition to glucose and acetate oxidation in neurons and glia in the rat OB at 9.4 T using a combination of ^{1}H MRS (metabolite quantification), J-edited ^{1}H MRS (GABA quantification), and ^{1}H-[^{13}C] MRS during intravenous infusions of [1,6-$^{13}C_2$]glucose (neuronal metabolism) or [2-^{13}C]acetate (astroglial metabolism).

METHOD: Sprague-Dawley rats (160-190g, fasted overnight) were anesthetized, tracheotomized, ventilated (69% N_2O/30% O_2/1% halothane), immobilized with tubocurarine-Cl, and femoral artery and veins cannulated. Rats received continuous infusions of either [1,6-$^{13}C_2$]glucose or [2-^{13}C]acetate intravenously for 2.5 hrs. Experiments were performed *in vivo* at 9.4 T (Bruker Instruments) using a surface coil positioned over the OB. Shimming was optimized using FASTMAP, typically resulting in water line widths of 14-16 Hz over the 15.6 uL (2.5x2.5x2.5 mm^3) volume-of-interest. GABA detection was achieved through J-difference editing (1). ^{1}H and ^{1}H-[^{13}C] MRS (2) spectra were acquired using a LASER-based localization method with adiabatic broadband decoupling during acquisition (TR/TE = 4000/12.5 ms), averaging over periods of 30 minutes to obtain time courses of spectra throughout the period of the measurement. Metabolites were quantified using a modified LCModel approach. Blood samples were taken periodically for the analysis of plasma glucose concentration and percent ^{13}C enrichment. At the end of the experiment, the brain was frozen *in situ* with liquid N_2. Metabolites were extracted from the frozen cortical tissue (3), and the concentrations and ^{13}C enrichments of glutamate, glutamine, and GABA were measured using ^{1}H-[^{13}C] MRS spectra of cortical extracts obtained at 11.74T (Bruker AM-500). Metabolic rates were evaluated by fitting a metabolic model with three compartments (3,4) (astroglia, glutamatergic and GABAergic neurons) using in-house CWave software to obtain simultaneous fits to the ^{13}C-glucose and ^{13}C-acetate infusion data.

RESULTS AND DISCUSSION: Fig. 1A shows the spectroscopic volume-of-interest in the rat OB. Figs. 1B/C show representative ^{1}H MR (TE = 68 ms) and GABA-edited ^{1}H MR spectra, the latter revealing the H2 and H4 methylene protons at 2.29 and 3.0 ppm, respectively. Fig. 1D shows a ^{1}H MR spectrum (TE = 12.5 ms) 120 mins following the onset of [2-^{13}C]acetate infusion, while Figs. 1E/F show ^{1}H-[^{13}C] NMR difference spectra after 120 mins of (E) [2-^{13}C]acetate and (F) [1,6-$^{13}C_2$]glucose infusion, respectively. The spectral resolution allows the visual separation of glutamate C4 and glutamine C4, whereas other resonances can be quantified by spectral fitting. Despite the small volume, GABA was readily detected. Metabolite line widths were 5-6 Hz for tCr and 4-5.5 Hz for NAA. Although the OB and the corresponding NMR volume (15.6 uL) were small, the combination of a high magnetic field, small surface coil (14 mm OD) and adiabatic pulses allowed the detection of ^{13}C level turnover in glutamate and glutamine following ^{13}C glucose or ^{13}C acetate infusions. The sensitivity also allowed the reproducible observation of absolute GABA concentration (4.17 ± 0.51 μmol/g (mean ± SD)). At 43%, the glial fraction of glutamate was much larger in the olfactory bulb than in the cortex. The rates of glutametergic neuron (V_{tcaGlu}), GABAergic neuron ($V_{tcaGABA}$) and glial (V_{tcaA}) TCA cycle fluxes were 0.55 ± 0.04, 0.15 ± 0.02 and 0.37 ± 0.03 respectively.On the other hand, the rates of glutamate-glutamine cycling ($V_{cyc(Glu/Gln)}$) and GABA-glutamine cycling ($V_{cyc(GABA/Gln)}$) were 0.37 ± 0.04 and 0.10 ± 0.01 μmol/g/min, with GABA synthesis (V_{GAD}) at 0.20 ± 0.03 μmol/g. GABA metabolism was notably greater than what has been reported in the cortex. Glutamate-glutamine cycling relative to GABA/glutamine cycling was less than the cortical values previously reported.

CONCLUSION: Compared to cortex in previous studies (2-4) the OB showed substantial metabolic differences including a higher levels of GABA and GABA/glutamine cycling and a larger astroglial glutamate pool size. The excellent spectral resolution and sensitivity for dynamic isotopic label experiments in the OB *in vivo* opens the way for studies of OB activation by odorants.

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