In vivo ^{19}F CSI of 2-fluoro-2-deoxy-D-glucose and 2-fluoro-2-deoxy-D-glucose-6-phosphate in rat brain

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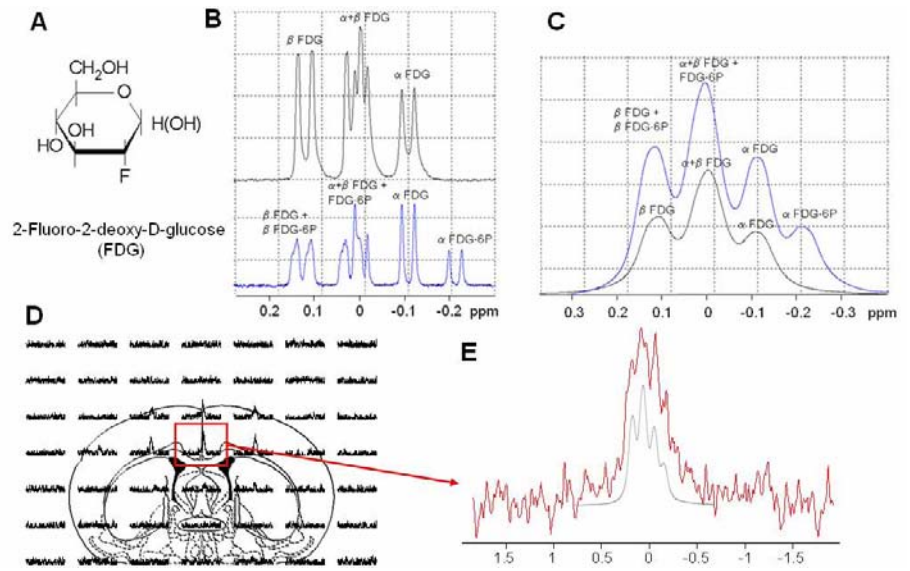
INTRODUCTION. ^{19}F MRS studies of 2-fluoro-2-deoxy-D-glucose (FDG) and 2-fluoro-2-deoxy-D-glucose-6-phosphate (FDG-6P) may be used for assessing tumor metabolism [1, 2]. The ^{19}F MRS measurements of FDG metabolism in the brain have either been achieved *ex vivo* from extracted tissue [3, 4] or *in vivo* by unusually long acquisition times (5 hours) [5]. However *in vivo* ^{19}F MRS studies in real-time (i.e., within an hour of start of FDG infusion) are rare primarily because of the low sensitivity due to low concentrations of FDG and FDG-6P in brain. Towards that end, here we report *in vivo* detection ^{19}F CSI data of FDG and FDG-6P in real-time in rat brain. Detection of FDG and FDG-6P by ^{19}F CSI within minutes of infusion represents a very important step towards exciting applications in assessing tumor pathophysiology.

METHODS. ***In vitro:*** Non-decoupled ^{19}F NMR spectra of two separate samples containing FDG and FDG-6P were acquired on an 11.7T Bruker vertical-bore spectrometer at 300K. The first sample contained 10mM FDG in 50% D_2O, the second contained 1mM FDG and 1mM FDG-6P in 50% D_2O. ***Animal preparation:*** Sprague-Dawley rats (n=2, 200-215 g) and Fisher rats (n=4, 170-220 g) were tracheotomized and artificially ventilated (70% N_2O, 30% O_2). 1 to 2 % halothane was used for induction during the animal preparation, and 0.3 to 0.7 % halothane was used for anesthesia. An intravenous line was used for administration of D-tubocurarine chloride or FDG (**A**). Various infusion times (10 to 20 minutes) were used such that the total infusion dose was between 200 and 1000 mg/Kg. An arterial line was used to monitor the animal physiology (blood pH, pO_2, pCO_2) throughout the experiment. ***Electrophysiology:*** The animals were placed in a stereotaxic holder on a vibration-free table inside a Faraday cage. Tiny burr holes above somatosensory cortex were made and tungsten microelectrodes were inserted at the depth of layer 4 with stereotaxic manipulators. The cerebral blood flow was measured using a bare fiber laser-Doppler probe. ***NMR:*** All ^{19}F NMR 2D-CSI data were obtained on a modified 11.7 T Bruker horizontal-bore spectrometer (Billerica, MA) using a ^{1}H/^{19}F resonator/surface probe using the following parameters: TR = 0.2 s, NS = 128, FOV = 3.2x3.2 cm, slice = 6 mm, acquisition time = 27 min; 8x8 encoding steps. All spectra were line broadened (10 Hz) and baseline corrected.



RESULTS AND DISCUSSION. Non-decoupled ^{19}F NMR spectrum of FDG (**B**; top) shows the existence of two different conformations, α and β. The presence of two non-equivalent ^{1}H nuclei adjacent to ^{19}F nucleus (**A**) results in splitting of each α and β resonances in four peaks (**B**; top). Non-decoupled ^{19}F NMR spectrum of the mixture of FDG and FDG-6P (**B**; bottom) indicate that the resonances of their β conformations are overlapping, while the two rightmost resonances of their α conformations are separated by 0.1 ppm (47 Hz) *in vitro*, and therefore well resolved. An increase in the linewidths of the NMR resonances for *in vivo* experiments at 11.7T by an additional 20 to 30 Hz was observed, due to the inherent inhomogeneities of the NMR sample (brain). Our *in vitro* results indicate that even with an additional line-broadening of 25 Hz, the resonances of two distinct chemical species FDG and FDG-6P can still be resolved (**C**). The ^{1}H decoupling of the ^{19}F spectrum was not used for these experiments for two reasons. First, the decoupling is accompanied by additional noise injection which partially negates the increase in the SNR introduced by the decoupling. Our *in vitro* results indicate a gain of less than 30% in SNR after decoupling (data not shown). Second, the use of a relatively short repetition time (TR=0.2s) might result in a large RF power deposition with decoupling, which is not desired for studies in brain metabolism. The short TR value with a low flip angle results in similar SNR compared to experiments with longer TR value with a larger flip angle. While the short TR value does not allow a complete relaxation of the ^{19}F magnetization, the effective signal increases due to a larger number of averages per each encoding step. The ^{19}F 2D CSI (**D,E**) shows the localized detection of both FDG and FDG-6P ^{19}F NMR resonances in real-time (27 minutes) *in vivo*. A comparison of *in vitro* and *in vivo* data acquired under exactly the same conditions allowed us to estimate that the FDG and FDG-6P concentrations in the brain are less than 1 mM, for a dose of 1000 mg/Kg of FDG infusion. These results explain the low SNR observed in our *in vivo* experiments. Also, this is most likely the reason that FDG was not directly detected previously in the brain in real-time. The effects of increasing the FDG infusion dose from 200 mg/Kg to 1000 mg/Kg on the rat physiology were determined by monitoring systemic blood pressure, cerebral blood flow, EEG and neuronal activity (multiunit activity and local field potential). The electrophysiological measurements indicate that there is no effect on rat basal neuronal activity for doses lower than 500 mg/Kg, while for doses higher than 500 mg/Kg the baseline neuronal activity is drastically reduced. Therefore, the optimal infusion dose required to obtain a good ^{19}F NMR signal without compromising the animal physiology is 500 mg/Kg, obtained by continuous infusion of FDG for ~10 min. In summary, in the present work we report for the first time the localized detection of FDG and FDG-6P ^{19}F CSI signals in real-time in rat brain. The localized detection of FDG and FDG-6P can allow determination of glucose metabolic rates.

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ACKNOWLEDGEMENTS. Supported in part by a gift from Pfizer and by grants MH-067528 and DC-003710 from NIH.