Redox Dependence and Compartmentation of [^{13}C]Pyruvate in the brain of deuterated rats bearing implanted C6 gliomas

T. B. Rodrigues[1], P. Lopez-Larrubia[1], and S. Cerdán[1]
[1]LISMAR, Instituto de Investigaciones Biomédicas CSIC, Madrid, Spain

Introduction. Pyruvate (pyr) plays a central role in cerebral metabolism, integrating glycolytic and oxidative responses and providing the carbon skeletons that couple the vital energy exchange between neurons and astrocytes. Intracellular pyruvate is normally produced from glucose (glc) in the cytosol of neural cells through the Embden-Meyerhoff pathway, or alternatively, it can be derived from extracellular monocarboxylates after transport to the cytosol. The relative contributions of these two pyruvate sources to the pyruvate pool and how the corresponding pyruvate precursors mix and interact intracellularly remain, however, less understood. Intracellular redox states are thought to play a dominant role in these processes. The modulation of glycolysis by the cytosolic redox state has been suggested to play a primary role in the glycolytic responses supporting glutamatergic neurotransmission (1,2). Moreover, reduced cytosolic redox has been demonstrated to decrease glycolytic flux in C6 cells (3) as well primary cultures of neurons (4). Similarly, two kinetically different pyruvate pools were described in neurons (4) and astrocytes (5) as well as in C6 cells (3). Nevertheless, no direct evidence was available, to our knowledge, on the modulation of the glycolysis by oxidized or reduced monocarboxylates, as well as on monocarboxylate compartmentation *in vivo*. In this work, we provide new evidences confirming the presence of at least two slowly exchanging pools of cytosolic pyr, and their sensitivity to redox changes, in different regions from the adult brain of rats bearing C6 tumors. In addition, we investigated the interaction between the pyruvate pools derived from [^{13}C]glc or [^{13}C]monocarboxylates by monitoring the exchange with ^{2}H of the methyl groups in the [^{13}C]lactate (lac) molecules derived from [^{13}C]glc or [^{13}C]monocarboxylates, respectively.

Methods. C6 gliomas were induced in Wistar rats by stereotaxic injection of C6 cells in the caudate nucleus. Ten days before the infusion, half of these animals received 50% $^{2}H_2O$ in the drinking water. Within the fourth week after injection, the rats were anesthetized with gaseous isofluorane and half of the animals that received deuterated water were infused in the left jugular vein with a solution of 0.2M of [1-^{13}C]glc and 0.4 M of [2-^{13}C]pyr (n=5), while the other half received an infusion of 0.2M of [1-^{13}C]glc and 0.4M of [U-$^{13}C_3$]lac (n=5), for 60 min. The same protocol was followed in the animals that received normal water in their diet (n=5, for each infusion conditions). Then, cerebral metabolism was arrested using a microwave fixation and dissected in three regions (right and left hemispheres and tumor) which were immediately frozen in liquid N_2. Extracts from the different regions of the same brain were prepared and analyzed by ^{13}C NMR spectroscopy (125.13MHz for ^{13}C).

Results and Discussion. Fig. 1A summarizes the relative contribution of glc to lac production in five rats bearing C6 tumors, as calculated from the singlet and the doublet lac C3 resonances, respectively. The relative contribution of [1-^{13}C]glc to [3-^{13}C]lac production decreased in the order contralateral (0.69±0.01%) > ipsilateral (0.58±0.05%) > tumor (0.48±0.04%). This suggests that the elevated lac levels present in the C6 glioma tumor and its surroundings are able to decrease significantly the glycolytic production of [3-^{13}C]lac from [1-^{13}C]glc. Fig. 1B illustrates the changes in the methyl (20.9 ppm) and methylene (69.3 ppm) resonances from lac after infusion of [1-^{13}C]glc and [2-^{13}C]pyr. In this case, the relative contributions of [1-^{13}C]glc or [2-^{13}C]pyr to cerebral lac production can be measured from the relative intensities of the lac C3 and C2 singlets. A similar glycolytic response to that shown in Fig. 1A is observed also in this case, indicating that infused pyr is rapidly transformed to lac extracerebrally *in vivo*, closely mimicking the effects of the lac and glc co-infusions. With these data, we are able to show that the increasing endogenous concentrations of lac in the glioma model decrease the glycolytic degradation of glc *in vivo*, confirming our previous findings on the modulation of glycolytic flux by the redox state in neurons or C6 glioma cells. Fig. 1C shows expansions of representative ^{13}C NMR spectra of the protonated and deuterated resonances of lac C2 and lac C3, as derived from infused mixtures of [2-^{13}C]pyr and [1-^{13}C]glc, in animals drinking 50% $^{2}H_2O$. The lac C3 resonance shows a pronounced singlet (s) derived from [3-^{13}C]lac, a shifted singlet (ss) derived from [3-^{13}C, 2-^{2}H]lac and a triplet (t) derived from [3-^{13}C, 3-^{2}H]lac. The lac C2 resonance shows an intense singlet (s) derived from [2-^{13}C]lac and a shifted singlet (ss) derived from [2-^{13}C, 3-^{2}H]lac. These resonances allow then determining the fractional monodeuteration in the methyl groups of [3-^{13}C] and [2-^{13}C]lac, respectively. The results show that the fractional monodeuteration of the methyl group from (2-^{13}C]lac molecules was always higher than that of [3-^{13}C]lac molecules in all regions investigated. For monodeuteration values close to 42% monodeuteration in [2-^{13}C]lac, the [3-^{13}C]lac methyl group became deuterated only to 29%. These results match well with our previous findings of pyr compartmentation in neurons and C6 glioma cells (3), providing now the first evidence, to our knowledge, on the compartmentation of the cerebral pyr pool *in vivo*.

Conclusion. Our results have shown that rats bearing C6 gliomas provide a convenient model to investigate the modulation of cerebral glycolytic flux by monocarboxylates in the contralateral, ipsilateral or tumor regions. We demonstrate inhibition of cerebral glycolysis by endogenous lac in the *in vivo* brain and disclose the *in vivo* compartmentation of the cerebral pyr pools derived from infused glc or monocarboxylates.

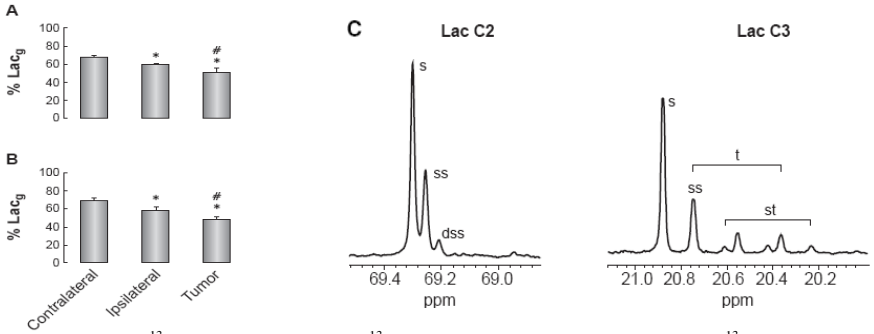

Figure 1: Relative contributions of [3-^{13}C]lac derived from [1-^{13}C]glc (Lac_g) relative to coinfused [U-$^{13}C_3$]lac (**A**) or to [2-^{13}C]pyr (**B**). * ($P < 0.05$) *vs.* contralateral hemisphere; $^{\#}$ ($P < 0.05$) *vs.* ipsilateral hemisphere. **C:** Representative lac C2 (69.3 ppm) and lac C3 (20.9 ppm) ^{13}C NMR regions, allowing the determination of the fractional deuteration in the methyl groups of [2-^{13}C] and [3-^{13}C]lac. s: singlet, ss: shifted singlet, dss: doubly shifted singlet, t: triplet, st: shifted triplet dss: doubly shifted singlet; Lac: lactate.

References: 1. Cerdán et al. Neurochem Int. 2006. 48:523. **2.** Ramirez et al. J Neurosci Res. 2007. 85:3244. **3.** Rodrigues et al. J Neurosci Res. 2005. 79:119. **4.** Cruz et al. J Neurosci Res. 2001. 66:771. **5.** Zwingmann et al. Glia. 2001. 34:200.