ONLINE SUPPLEMENT

Pyruvate Protects Brain Against Ischemia-Reperfusion Injury by Activating Erythropoietin Signaling Pathway

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Research support: This work was supported by National Institutes of Health grants R01NS054687 (SY), R01NS054651 (SY), R01NS076975 (RM) and T32 AG020494 (MR).

Running title: Pyruvate-Induced Cerebroprotection

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Supplement materials and methods

Immunoblot of hypoxia-responsive proteins. Contents of HIF-1α, EPO, Akt/pAkt, and actin were analyzed by immunoblotting of brain extracts and/or cell lysates. Primary antibodies were mouse monoclonal antibodies against HIF-1α, EPO, Akt/p-Akt, and actin (Santa Cruz Biotechnology). Goat anti-mouse secondary antibody was obtained from Jackson Immunoresearch (West Grove, PA). Protein bands were quantified (Ultraviolet Products, Upland, CA) and normalized to actin.

EPO pathway inhibition. To interrogate the involvement of EPO signaling in the pyruvate cytoprotection, soluble EPO receptor (sEPOR, Sigma) was applied to C6 glial cells (n=10/group) cells. Cells were pretreated for 6h with sEPOR. After H/R, cell viability was determined with calcein AM assay.

Supplemental Figures

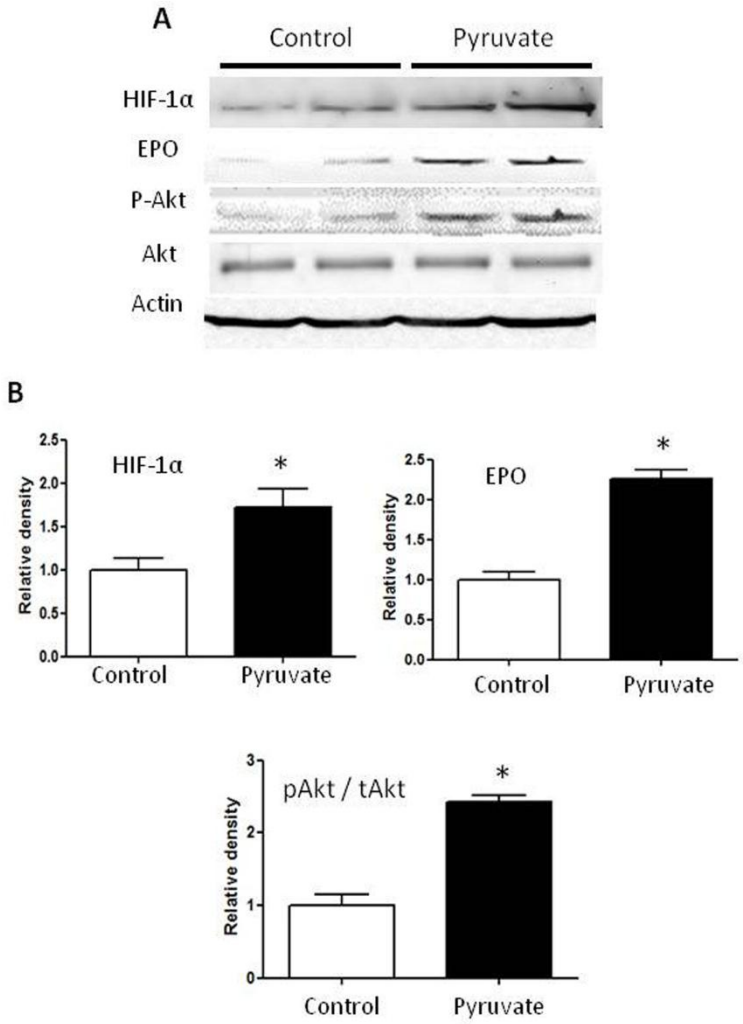

Figure S1. HIF-1α, EPO, and pAkt/Akt protein contents in C6 glioma cells. **A)** 10 mM pyruvate increased expression of HIF-1α, EPO, and EPOR, in parallel with Akt activation. **B)** Immunoblot band densities in pyruvate-treated cells were normalized to respective controls (*P<0.05 *vs*. Control).

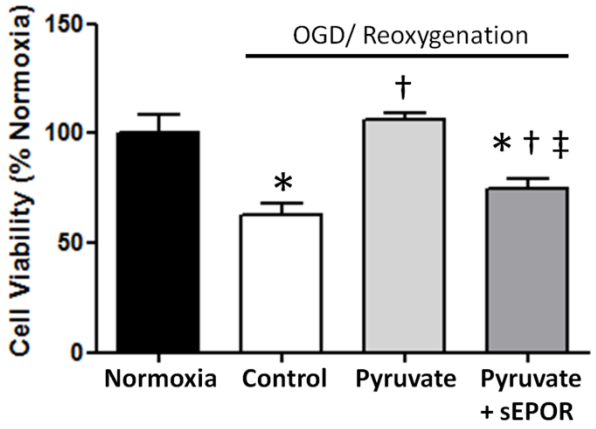

Figure S2. Effect of suppression of EPO signaling pathway on cell viability. Interruption of EPO pathway with soluble EPOR dampened pyruvate-induced protection in C6 glioma cells. *P<0.05 *vs.* Normoxia; †P< 0.05 *vs.* Control; ‡P< 0.05 *vs.* Pyruvate.

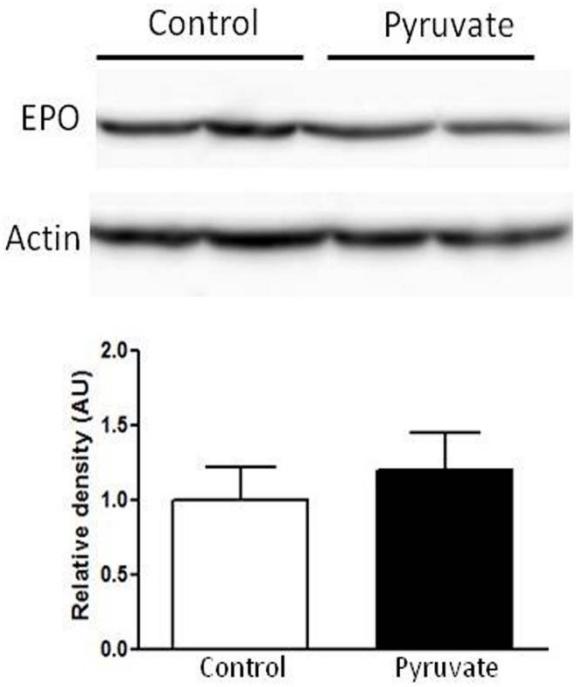

Figure S3. Effect of pyruvate infusion on peripheral EPO protein content. Renal EPO protein content was not significantly increased as compared to NaCl infused control group.