# Pyruvate preserves antiglycation defenses in porcine brain after cardiac arrest

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## Impact statement

Recent studies have demonstrated a pivotal role of protein glycation in brain injury. Methylglyoxal, a by-product of glycolysis and a powerful glycating agent in brain, is detoxified by the glutathione-catalyzed glyoxalase (GLO) system, but the impact of cardiac arrest (CA) and cardiocerebral resuscitation (CCR) on the brain's antiglycation defenses is unknown. This study in a swine model of CA and CCR demonstrated for the first time that the intense cerebral ischemia–reperfusion imposed by CA-resuscitation disabled glyoxalase-1 and glutathione reductase (GR), the source of glutathione for methylglyoxal detoxification. Moreover, intravenous administration of pyruvate, a redox-active intermediary metabolite and antioxidant in brain, prevented inactivation of glyoxalase-1 and GR and blunted protein glycation in cerebral cortex. These findings in a large mammal are first evidence of GLO inactivation and the resultant cerebral protein glycation after CA-resuscitation, and identify novel actions of pyruvate to minimize protein glycation in postischemic brain.

## Abstract

Cardiac arrest (CA) and cardiocerebral resuscitation (CCR)-induced ischemia–reperfusion imposes oxidative and carbonyl stress that injures the brain. The ischemic shift to anaerobic glycolysis, combined with oxyradical inactivation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), provokes excessive formation of the powerful glycating agent, methylglyoxal. The glyoxalase (GLO) system, comprising the enzymes glyoxalase 1 (GLO1) and GLO2, utilizes reduced glutathione (GSH) supplied by glutathione reductase (GR) to detoxify methylglyoxal resulting in reduced protein glycation. Pyruvate, a natural antioxidant that augments GSH redox status, could sustain the GLO system in the face of ischemia–reperfusion. This study assessed the impact of CA-CCR on the cerebral GLO system and pyruvate's ability to preserve this neuroprotective system following CA. Domestic swine were subjected to 10 min CA, 4 min closed-chest CCR, defibrillation and 4 h recovery, or to a non-CA sham protocol. Sodium pyruvate or NaCl control was infused (0.1 mmol/kg/min, intravenous) throughout CCR and the first 60 min recovery. Protein glycation, GLO1 content, and activities of GLO1, GR, and GAPDH were analyzed in frontal cortex biopsied at 4 h recovery. CA-CCR produced marked protein glycation which was attenuated by pyruvate treatment. GLO1, GR, and GAPDH activities fell by 86, 55, and 30%, respectively, after CA-CCR with NaCl infusion. Pyruvate prevented inactivation of all three enzymes. CA-CCR sharply lowered GLO1 monomer content with commensurate formation of higher molecular weight immunoreactivity; pyruvate preserved GLO1 monomers. Thus, ischemia–reperfusion imposed by CA-CCR disabled the brain's antiglycation defenses. Pyruvate preserved these enzyme systems that protect the brain from glycation stress.

**Keywords:** Glutathione, glycation, glyoxalase-1, methylglyoxal, nuclear factor erythroid 2-related factor 2, swine

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## Introduction

Cardiac arrest (CA) is the leading cause of death in the United States and worldwide. Fewer than 10% of CA victims survive to hospital discharge, and many survivors suffer persistent neurocognitive deficits due to severe ischemia–reperfusion damage to the brain.[1] The mechanisms of brain injury and cognitive impairment are complex and poorly delineated, so effective treatments remain elusive. Cardiocerebral resuscitation (CCR) and effective cardiac defibrillation reperfuse the ischemic brain, but reperfusion ignites an explosive burst of reactive oxygen, nitrogen and dicarbonyl formation which intensifies intracellular $Ca^{2+}$ overload, glutamate excitotoxicity and inflammation, culminating in extensive neuronal death.[2] However, numerous clinical trials failed to demonstrate appreciable neurocognitive benefits of antioxidant and anti-inflammatory interventions after CA and CCR.[3,4]

The powerful glycating dicarbonyl methylglyoxal, a product of spontaneous decomposition of the glycolytic intermediates glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), has been implicated in the pathogenesis of Alzheimer's Disease, Parkinson's Disease, and other neurodegenerative disorders.[5,6] Methylglyoxal accumulates when glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is disabled by reactive oxygen intermediates, causing the upstream glycolytic intermediates GAP and DHAP to accumulate.[7,8] Methylglyoxal glycates, nucleic acids, and proteins, generating advanced glycation endproducts.[9] Glycation of arginine and lysine residues by methylglyoxal disables enzymes of intermediary metabolism, e.g. GAPDH,[10,11] aconitase,[12] and glutamate dehydrogenase,[13] as well as endogenous antioxidant defense, including superoxide dismutase,[14,15] glutathione peroxidase,[10,12] glutathione *S*-transferase,[15] glutathione reductase (GR),[10,12] and glucose 6-phosphate dehydrogenase (G6PDH).[15] GR transfers electrons from NADPH to glutathione disulfide (GSSG), thereby maintaining glutathione (GSH) content and GSH/GSSG redox state without requiring *de novo* synthesis. Thus, interventions that reduce formation or promote degradation of methylglyoxal may protect crucial enzymes from carbonyl stress and, thereby, minimize post-CA brain injury.

Catabolism of methylglyoxal to a non-toxic product, D-lactate, is catalyzed by GSH and the sequential actions of glyoxalase 1 (GLO1) and glyoxalase 2 (GLO2).[16] Oxyradicals generated in ischemic and postischemic brain deplete GSH and, thus, disable the methylglyoxal-detoxifying glyoxalase (GLO) system. Interventions that augment GSH may promote GLO-catalyzed methylglyoxal catabolism.[17]

Pyruvate, a natural intermediary metabolite, antioxidant, and energy substrate, has proven to be a powerful neuroprotectant in numerous animal models of brain injury. Pyruvate protected rat brain from bilateral carotid artery occlusion,[18] cortical contusion injury,[19] closed head injury,[20] and kainate-induced seizures.[21] In a swine model of hemorrhagic shock, intravenous resuscitation with pyruvate suppressed excitotoxic glutamate release within the cerebral cortex and stabilized cortical electrical activity.[22] In dogs subjected to CA and resuscitation, pyruvate minimized neuronal death and caspase-3 activity in the hippocampal CA1 subregion, prevented degeneration of cerebellar Purkinje cells, and sharply lowered neurological deficit 24 and 48 h postarrest.[23] Because pyruvate augments GSH/GSSG[24] and readily traverses the blood–brain barrier,[25] it is conceivable that pyruvate may enhance the GSH-dependent methylglyoxal-detoxifying GLO system. Accordingly, this study tested the hypothesis that pyruvate-enriched resuscitation of swine following CA can promote methylglyoxal detoxification and preserve activity of the methylglyoxal-vulnerable enzymes GLO1, GR, and GAPDH. To our knowledge, this study is the first to directly evaluate modulation of the GLO system in the context of CA-CCR.

## Methods

### Animals and surgical procedures

Animal experimentation was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center (protocol # 2012/13-29-A10) and was conducted in accordance with the *Guide to the Care and Use of Laboratory Animals* (U.S. National Research Council publication 85-23, revised 2011). Twenty Yorkshire swine of either sex weighing 25–35 kg were randomly assigned to one of three experimental groups: (1) sham protocol ($n=6$) without CA, (2) CA-CCR with NaCl infusion ($n=7$), or (3) CA-CCR with Na-pyruvate infusion ($n=7$).

After an overnight fast, pigs were premedicated with telazol (6.7 mg/kg *im*) and xylazine (1.3 mg/kg *im*), intubated, and maintained under a surgical plane of anesthesia by mechanical ventilation with 1–3% isoflurane in 100% $O_2$. Cannulas were placed in the left femoral artery via inguinal incision to monitor arterial pressure and sample arterial blood, and in the right jugular vein via cervical incision to sample cerebrovascular venous blood and infuse treatments. Surface electrodes were applied to monitor cardiac rhythm by lead II electrocardiography, and heparin (300 U/kg) was injected intravenous.

### CA

Swine were subjected to CA, CCR, defibrillation, and recovery.[26] A pacing wire was inserted into the jugular venous cannula and advanced until it contacted the right ventricular endocardium, and the heart was arrested by pacing at 4 Hz. After 10 min CA, precordial compressions were administered at a rate of 100/min for 4 min, producing cerebral perfusion pressure of $69 \pm 5$ mmHg.[26] Transthoracic countershocks (200–300 J) were applied singly, with 30 s intervening CCR, until spontaneous cardiac electromechanical rhythm was reestablished. Sodium pyruvate or NaCl was infused intravenous (0.1 mmol/kg/min) during CCR and the first 60 min of recovery.

### Plasma analysis

Blood samples were sedimented by centrifugation, and plasma supernatants were extracted as described previously.[27] Pyruvate concentrations in plasma extracts were measured by colorimetric assays[28] in a Shimadzu Instruments model UV-1800 spectrophotometer.

### Cerebrocortical lysates

At 4 h recovery, the heart was rearrested and then the brain was cross-perfused with 6 L of ice-cold 0.9% NaCl to flush the cerebral circulation. The frontal cortex was excised; the pia mater, arachnoid, and superficial vasculature were removed; and cortical tissues were minced to <5 mm fragments and pulverized in a mortar in ice-cold lysis buffer (50 mM Tris–HCl, 2 mM, EDTA, protease inhibitor cocktail, 2 mM phenylmethylsulfonyl fluoride, pH 7.4). After bringing the volume to 15 ml with lysis buffer, homogenates were

vigorously vortexed for 90 s to release intracellular and intramembraneous proteins, and centrifuged (c. $4000g_{max}$) at 4°C for 30 min. Supernatant aliquots were stored in Eppendorf minivials at −20°C until analysis.

### Cerebrocortical enzyme activities and protein content

Enzyme activities were assayed at 37°C. GLO1 activity in frontal cortex lysates was assayed spectrophotometrically[29] by monitoring the appearance of *S*-D-lactoylglutathione at 240 nm ($\varepsilon = 2.86$ mM/cm) in a Shimadzu Instruments model UV-1800 spectrophotometer. A lysate-free reaction mixture containing hemithioacetal substrate exhibited no intrinsic reaction. GLO2 activity was assessed from the rate of disappearance of *S*-D-lactoylglutathione.[29] GR activity was determined from the rate of disappearance of NADPH,[30] G6PDH activity from the rate of NADPH formation,[31] and GAPDH from the rate of NADH formation[32] monitored at 339 nm ($\varepsilon = 6.22$ mM/cm). Protein concentrations in homogenates were measured by Bradford's colorimetric assay[33] in a 96-well microplate spectrophotometer (Biotek Powerwave XS). Enzyme activities are reported as units per milligram protein, where one unit equals conversion of 1 μmol substrate to product per min.

### Immunoblotting

Cortical lysates were analyzed by electrophoresis and immunoblotting to detect GLO1 and argpyrimidine, which is produced by methylglyoxal glycation of arginyl residues. Proteins (30 μg/lane) were electrophoretically separated on 4–15% SDS-polyacrylamide gels (BioRad, Hercules, CA) and electrophoretically transferred (22 V for 16 h at 4°C) to vinyl membranes (BioRad). Membranes were blocked with 5% non-fat milk (BioRad) in Tris-buffered saline with Tween (TTBS) for 1 h at room temperature, incubated overnight at 4°C with primary antibodies against argpyrimidine (mouse monoclonal; NOF-N213430-EX, Cosmobio, Tokyo, Japan), or GLO1 (rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA), washed three times with TTBS, and then incubated with horseradish peroxidase-conjugated secondary antibodies (goat anti-mouse IgG for argpyrimidine and goat anti-rabbit IgG for GLO1; Cayman Chemical, Ann Arbor, MI) for 1 h at room temperature. Membranes were then washed three times with TTBS and immunoreactivity was detected with SuperSignal West Pico chemiluminescent substrate (Thermo Scientific, Rockford, IL). To normalize GLO1 and argpyrimidine immunoblots for protein loading, membranes were stripped and reprobed with primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) against β-tubulin or β-actin, respectively, and subsequent horseradish peroxidase-conjugated secondary antibodies (Cayman Chemical) as described above. Band densities were captured with ChemDoc Image Lab, version 5.2.1, (BioRad) system and quantified by use of the Image Studio program (LI-COR), version 5.0.21.

### Statistical analyses

Enzyme activities and immunoblot values, expressed as means ± SEM, were compared by single-factor ANOVA. Plasma pyruvate concentrations were compared by two-factor (treatment, time) ANOVA, with repeated measures on time. Tukey's HSD *post hoc* analyses were applied when ANOVA revealed statistically significant effects. Statistical analyses were accomplished with SigmaStat v10. Values of $P < 0.05$ were taken to indicate statistically significant effects.

## Results

### Pyruvate infusion increased arterial pyruvate concentration during postarrest recovery

Pyruvate infusion (0.1 mmol/kg/min, intravenous) during 4 min CCR and the first 60 min following recovery of spontaneous circulation (ROSC) raised arterial pyruvate concentration from 0.15 ± 0.03 to 3.0 ± 0.4 mM by 60 min ROSC (Figure 1). Arterial pyruvate fell after infusion to 0.54 ± 0.33 mM by 4 h ROSC. In the NaCl-infused pigs, plasma pyruvate concentration rose modestly, from 0.12 ± 0.02 mM prearrest to 0.37 ± 0.12 and 0.33 ± 0.19 mM at 60 min and 4 h ROSC, respectively (Figure 1).

### CA-CCR depleted and pyruvate preserved cerebrocortical GLO1 monomer (21 kDa) content

CA/CCR produced marked reduction in anti-GLO1 immunoreactivity at c. 21 kDa (Figure 2(a) and (b)), the molecular mass of GLO1 monomers,[34] and increased immunoreactivities at higher molecular masses coinciding with GLO1 dimers (42 kDa) and larger (c. 70 and 150 kDa), unidentified immunoreactivities (Figure 2(a)). Pyruvate treatment preserved cerebrocortical GLO1 monomer content after CA-CCR (Figure 2(b) and (c)), yet the higher molecular mass immunoreactivities were still evident (Figure 2(a)).

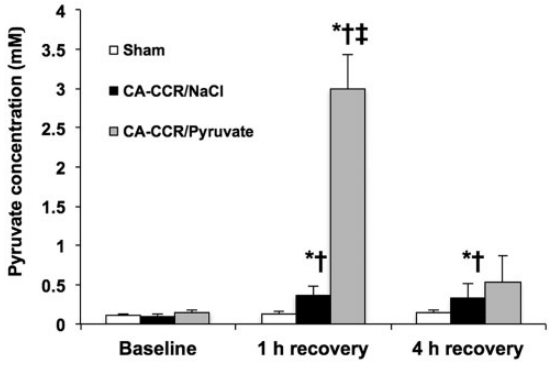


**Figure 1** *Plasma pyruvate concentrations.* Pyruvate was measured in plasma extracts collected at pre-CA baseline, 1 h post-CA recovery (i.e. the end of intravenous NaCl or sodium pyruvate infusion), and at 4 h recovery (i.e. 3 h postinfusion), and at the analogous times in non-arrested sham experiments. Mean values ± SEM from six sham, seven CA-CCR + NaCl, and seven CA-CCR + pyruvate experiments. *$P < 0.05$ versus baseline of the same group; †$P < 0.05$ versus sham; ‡$P < 0.05$ versus NaCl treated. CA: cardiac arrest; CCR: cardiocerebral resuscitation

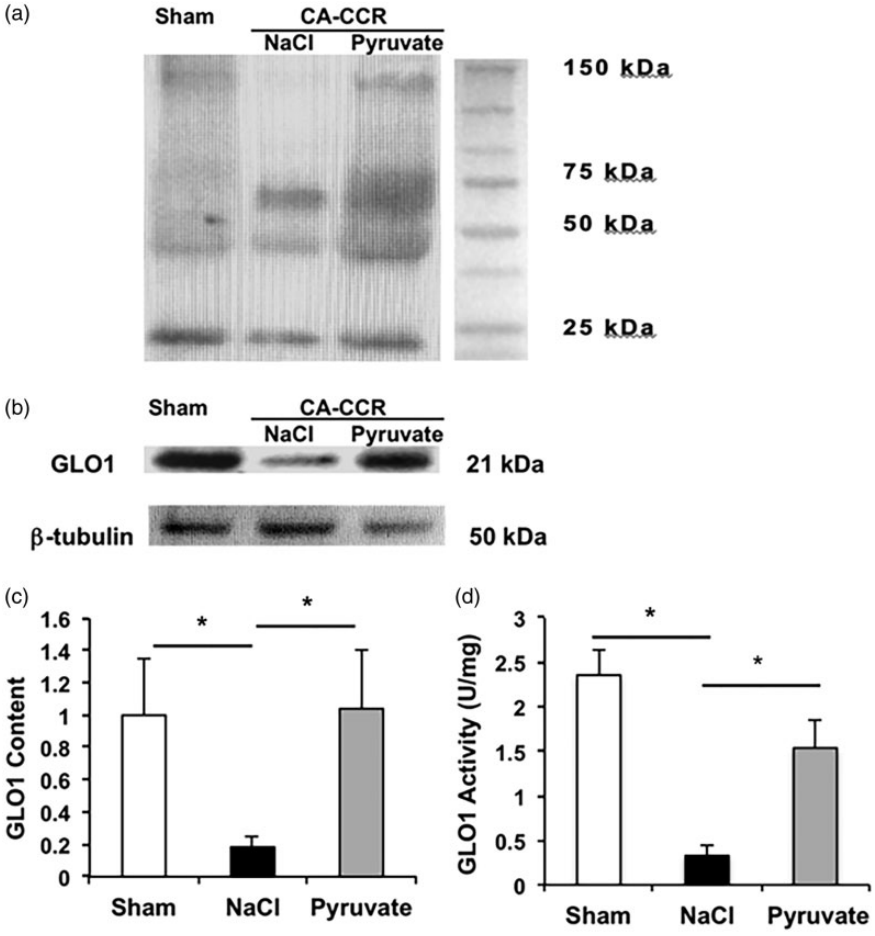


**Figure 2** *Glyoxalase 1 (GLO1) in cerebrocortical lysates.* (a) GLO1 protein content was assessed in cerebrocortical homogenates from each treatment group ($n = 4$) obtained at 4 h postarrest recovery. After electrophoretic separation, proteins were transferred to nylon membranes and incubated with antibodies against GLO1 monomer (21 kDa). Immunoreactivities at higher molecular masses may represent GLO1 oligomers or complexes with other proteins. (b) GLO1 monomer immunoreactivity at 21 kDa. Band densities were normalized to the relative signal intensity of β-tubulin loading control. (c) Relative content of GLO1 monomer bands at 21 kDa normalized to beta-tubulin. (d) Activities (U/mg protein) of GLO1 were measured in lysates of frontal cortex harvested at 4 h post-CA recovery, and normalized to total protein contents of the same lysates. (c, d): mean values ± SEM from four experiments per group. Indicated comparisons: *$P < 0.05$. CA: cardiac arrest; CCR: cardiocerebral resuscitation

### Pyruvate infusion preserved cerebrocortical GLO1 activity

In the NaCl-infused pigs, cerebrocortical activity of the principal methylglyoxal-detoxifying enzyme GLO1 fell by 86% ($P < 0.01$) versus sham (Figure 2(d)). In contrast, pyruvate infusion protected GLO1, increasing the enzyme activity 4.6-fold versus NaCl infusion ($P < 0.01$) to 65% of GLO-1 activity in the non-arrested sham group ($P = 0.56$). Thus, CA-CCR disabled GLO1, but pyruvate treatment largely preserved methylglyoxal-detoxifying GLO1 activity.

### Impact of CA-CCR and pyruvate on cerebrocortical GLO2 activity

In the NaCl-infused pigs, cerebrocortical activity of the secondary GLO system enzyme GLO2 was unaltered by CA-CCR versus sham (Figure 3(a)). Pyruvate treatment unexpectedly lowered GLO2 activity by 47% versus NaCl-infused CA-CCR and by 43% versus sham ($P < 0.05$).

### Pyruvate treatment augmented cerebrocortical GR activity

Cerebrocortical activity of the critical antioxidant enzyme GR fell by 55% ($P < 0.05$) in the NaCl-infused post-CA-CCR versus non-arrested sham pigs (Figure 3(b)). Pyruvate sharply increased GR activity by sixfold versus NaCl ($P < 0.001$), to a value 2.4-fold higher than sham ($P < 0.05$). Thus, CA-CCR inactivated the pivotal antioxidant enzyme GR, yet pyruvate treatment profoundly increased cerebrocortical GR activity versus NaCl control.

### Pyruvate protected cerebrocortical GAPDH after CA

Cerebrocortical GAPDH activity fell by 30% following CA-CCR with NaCl infusion during CCR and the first 60 min recovery versus non-arrested sham controls ($P < 0.05$). However, sodium pyruvate infusion preserved GAPDH activity (Figure 3(c)). Neither CA/CCR nor pyruvate treatment modulated the activity of G6PDH, a pivotal source of NADPH in brain (Figure 3(d)).

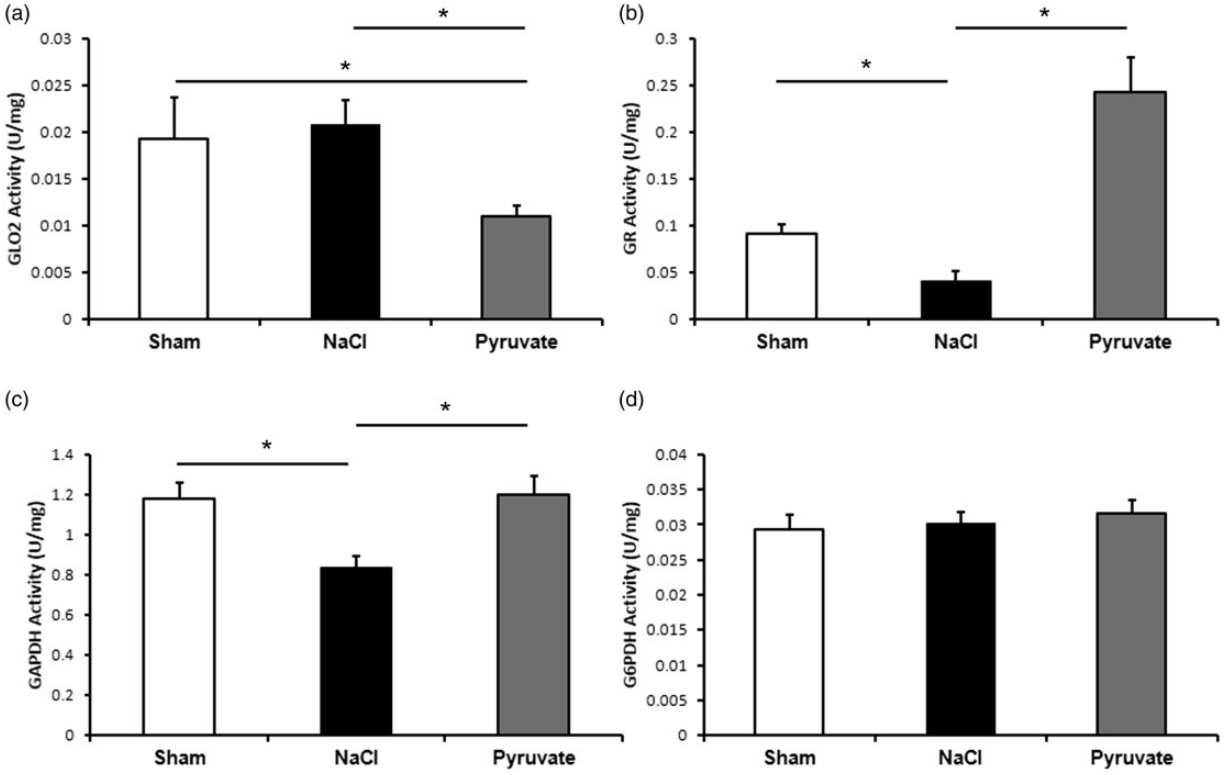


**Figure 3** *Cerebrocortical enzyme activities*. Enzyme activities (U/mg of protein) of glyoxalase 2 (GLO2: (a)), glutathione reductase (GR: (b)), glyceraldehyde-3-phosphate dehyrogenase (GAPDH: (c)) and glucose-6-phosphate dehydrogenase (G6PDH: (d)) were measured in lysates of frontal cortex harvested at 4 h post-CA recovery, and normalized to total protein contents of the same lysates. Mean values ± SEM from six sham, seven CA-CCR + NaCl infusion, and seven CA-CCR + pyruvate infusion experiments. Indicated comparisons: *$P < 0.05$. CA: cardiac arrest; CCR: cardiocerebral resuscitation

### Pyruvate infusion dampened post-CA protein glycation in swine cerebral cortex

Argpyrimidine, a product of irreversible methylglyoxal glycation of arginyl residues, was detected by immunoblot of cerebrocortical extracts harvested 4 h after CA-CCR (Figure 4(a)). Compared to sham, intense immunolabeling over a broad range of electrophoretic mobilities was evident in cerebral cortex after CA with NaCl infusion during CCR and the first 60 min postarrest (Figure 4(a)), indicating marked protein glycation. Immunoreactivity was prominent at c. 65 kDa, which approximates the molecular mass of occludin, a major component of the adherent junctions between cerebrovascular endothelial cells[35] and a target of methylglyoxal glycation.[36] Pyruvate infusion attenuated argpyrimidine immunoreactivity. Thus, pyruvate blunted CA-CCR-induced protein glycation in porcine cerebral cortex (Figure 4(b)).

## Discussion

In this study the ischemia–reperfusion stress imposed by CA and CCR disabled the brain's antiglycation defenses. Specifically, the CA-resuscitation protocol inactivated GLO1, the rate-controlling enzyme of the methylglyoxal-detoxifying GLO system[37] and GR, which maintains redox state of GSH, an essential substrate and catalyst of the GLO system.[16] By disabling these enzymes, CA-CCR compromises cellular defenses against glycation stress, potentially culminating in irreversible injury of neurons, astroglia, and other brain cells. However, intravenous infusion of pyruvate, a natural intermediary metabolite, energy substrate, and antioxidant,[38] maintained activities of critical enzyme components of the antiglycation machinery and dampened protein glycation. In addition to its known energy-yielding, antioxidant, and anti-inflammatory properties,[38] pyruvate's protection of the brain's antiglycation defenses (Figure 5) represents a novel cytoprotective mechanism of this pluripotent metabolite.

### Inactivation of GR and GLO1 by cerebral ischemia–reperfusion

GR transfers electrons from NADPH to GSSG to regenerate GSH, thereby maintaining the principal intracellular antioxidant and GLO catalyst.[16] GLO1 catalyzes the rate-controlling step in the methylglyoxal-detoxifying GLO cycle. Thus, the partial inactivation of GR and the more profound inactivation of GLO1 following CA-CCR may threaten the brain's antioxidant and antiglycation defenses (Figure 5). GR is inactivated by methylglyoxal[12,15] and GLO1 by *S*-glutathionylation secondary to oxidative stress.[39] Thus, reactive oxygen species produced in the ischemic and postischemic brain could intensify carbonyl stress in at least three ways: (1) increased utilization of GSH to detoxify oxyradicals, thereby depleting the essential GLO co-factor; (2) inactivation of GR, thereby impeding GSH regeneration; and (3) GAPDH inactivation, causing

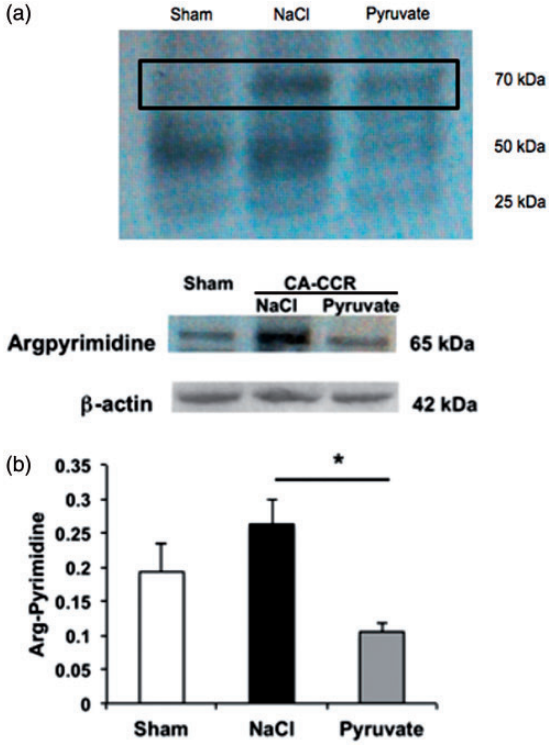


**Figure 4** *Immunoblot detection of protein glycation.* (a) Protein glycation was assessed in cerebrocortical lysates obtained at 4 h post-CA recovery. After electrophoretic separation, proteins were transferred to nylon membranes and incubated with antibodies against the major glycation product, argpyrimidine. The rectangle within the upper immunoblot circumscribes the c. 65 kDa bands which co-migrate with occludin standards. The c. 65 kDa immunoreactivity from a second immunoblot, and β-actin loading control, are shown below.
(b) Densitometry of the c. 65 kDa bands relative to β-actin (*$P < 0.05$). Mean values ± SEM, four experiments per group. CA: cardiac arrest

accumulation of the methylglyoxal precursors DHAP and GAP and, thus, intensifying methylglyoxal formation.

### Inactivation of GAPDH: Potential mechanism of methylglyoxal accumulation in postischemic brain

The predominant source of methylglyoxal is the spontaneous dephosphorylation of the glycolytic intermediates DHAP and GAP.[11] These compounds, generated by aldolase-catalyzed cleavage of fructose 1,6-*bis*-phosphate, lie immediately upstream of GAPDH in the glycolytic sequence. GAPDH inactivation causes DHAP and GAP to accumulate and form methylglyoxal, especially under conditions that augment glycolysis, e.g. ischemia–reperfusion. Several factors associated with ischemia–reperfusion may conspire to inactivate GAPDH and promote methylglyoxal formation. During ischemia, the lack of oxygen and resultant failure of mitochondrial respiration causes NADH accumulation and concomitant $NAD^+$ depletion in the cytosol, depriving GAPDH of its essential co-factor. GAPDH is particularly susceptible to chemical modification due to the presence of highly reactive cysteine, lysine, and arginine residues at its catalytic domain.[40,41] The enzyme is inactivated by *S*-glutathionylation;[42,43] moreover, GAPDH is itself a glycation target,[10,11] and as methylglyoxal accumulates a feed-forward cycle of GAPDH inactivation (Figure 5) may ensue.

### Pyruvate intervention for carbonyl stress

Pyruvate's diverse metabolic and antioxidant properties may support multiple neuroprotective mechanisms. As an α-keto carboxylate, pyruvate reduces reactive oxygen and nitrogen species (RONS) to non-reactive products in direct, non-enzymatic reactions.[24] Also, conversion of pyruvate to oxaloacetate by pyruvate carboxylase, an enzyme active in astrocytes,[44] may generate enough citrate to inhibit phosphofructokinase,[45] which could potentially divert glycolytic flux into the hexose monophosphate pathway, the major source of NADPH to maintain GSH.[46] The resultant increase in glucose 6-phosphate content could possibly augment hexose monophosphate pathway flux and NADPH production, despite the lack of impact of CA-CCR or pyruvate on the intrinsic G6PDH activity.

In addition to controlling GAPDH-inactivating RONS, pyruvate also restores cytosolic $NAD^+$ via the lactate dehydrogenase reaction, thereby providing the essential co-factor to sustain GAPDH flux;[47] this effect, combined with antioxidant protection of the enzyme by pyruvate and GSH, could minimize GAP and DHAP accumulation and, thus, methylglyoxal formation (Figure 5). Pyruvate also is a readily oxidized metabolic fuel which could hasten recovery of cellular energy reserves following reperfusion and reoxygenation of brain.[22]

Pyruvate may afford a ready intervention to protect the brain from methylglyoxal-induced glycation stress. Because pyruvate is highly soluble in aqueous solution, highly concentrated (e.g. 2 M) pyruvate solutions are readily prepared, thereby minimizing the infusion volume necessary to attain therapeutically effective pyruvate concentrations in the systemic circulation. Importantly, pyruvate traverses the blood–brain barrier via monocarboxylate transporters in the cerebrovascular endothelium,[25,48] delivering the compound to the brain parenchyma. The plasma membranes of neurons[49] and astrocytes[50] also harbor monocarboxylate transporters, delivering pyruvate to the cell interior where oxidative and carbonyl stress originates.

### Limitations

Several limitations of this study must be acknowledged. Although immunoblots revealed extensive protein glycation, specifically argpyrimidine adducts, after CA-resuscitation, and marked attenuation of protein glycation by pyruvate treatment, the specific glycated proteins have not been identified. Similarly, the higher molecular weight proteins or complexes showing GLO1 immunoreactivity are as yet unidentified. The enzymes were analyzed in whole brain homogenates, and the impact of CA-CCR and pyruvate on the specific cell types that comprise the histologically complex brain have not been delineated. Glycolysis is a major energy source in astrocytes, whereas neurons rely primarily on oxidation of lactate supplied by adjacent astrocytes,[51] so GAPDH inactivation may impact astrocytes directly and neurons indirectly. GSH metabolism is similarly compartmented: the bulk of the GSH is

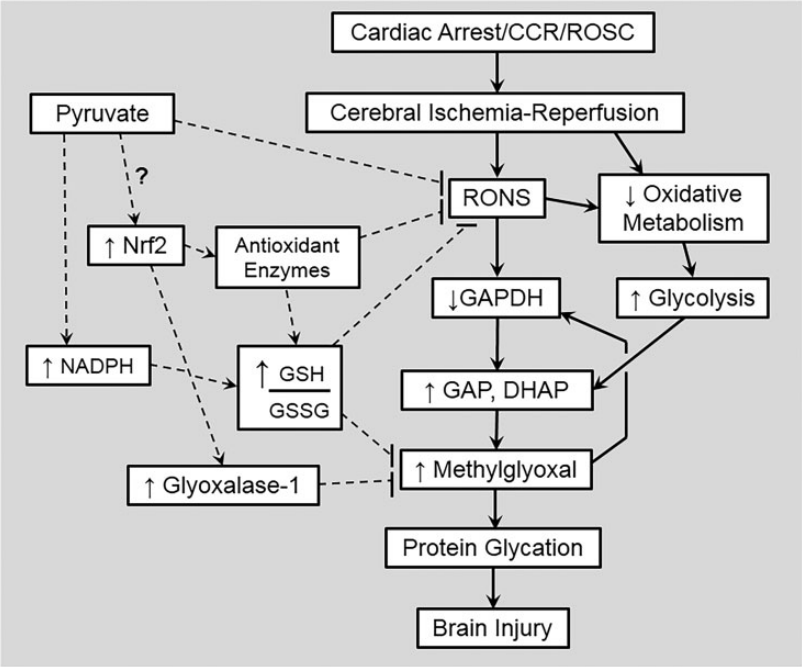


**Figure 5** *Proposed mechanisms of cerebral ischemia–reperfusion induction of carbonyl stress and of pyruvate's anticarbonyl actions.* RONS generated during cerebral ischemia–reperfusion inactivate metabolic enzymes and, thus, compromise oxidative ATP production, thereby activating glycolytic flux proximal to GAPDH, while inactivating GAPDH. The resultant accumulation of the methylglyoxal precursors, GAP and DHAP, provokes methylglyoxal accumulation and protein glycation which are intensified by concomitant inactivations of glutathione reductase (GR) and the methylglyoxal-detoxifying enzyme, glyoxalase-1 (GLO1). Pyruvate intervention dampens protein glycation by directly detoxifying RONS, preserving GLO1 and GR activities, and possibly activating Nrf2-driven gene expression which contributes to augmentation of antioxidant and methylglyoxal-detoxifying enzymes including GLO1. Broken lines and arrows represent pyruvate's actions. DHAP: dihydroxyacetone phosphate; GAP: glyceraldehyde 3-phosphate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; RONS: reactive oxygen and nitrogen species

generated in astrocytes,[52] released into the extracellular space,[53] and degraded into its constituent amino acids which are taken up by neurons and reassembled into GSH.[54]

The study's major biochemical endpoints were examined at a single time point, 4 h after cardioversion and ROSC. The longer term responses of the glycolytic, antioxidant, and anticarbonyl machinery to CA-CCR and pyruvate, and their neurobehavioral consequences, remain to be evaluated. In this regard, it should be noted that the pyruvate derivative ethyl pyruvate has been found to stabilize the master antioxidant response transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), which acts upon antioxidant response elements in upstream promoter regions of a host of antioxidant and anti-inflammatory genes.[53,55,56] GSH-synthesizing enzymes,[53,57,58] GR, and GLO1[59] are products of the Nrf2 gene program. Whether this Nrf2 mechanism is activated by non-ethylated pyruvate (Figure 5) remains to be determined. Pyruvate infusion lowered arterial free (unbound) $Ca^{2+}$ from c. 1.4 to c. 1.0 mM (data not shown), an effect that may be due in part to pyruvate-induced alkalemia.[26] Pyruvate-induced hypocalcemia may limit protracted pyruvate treatment in clinical settings.

## Conclusions

This study demonstrated for the first time that acute cerebral ischemia–reperfusion imposed by CA and resuscitation in swine inactivates GR and GLO1, key components of the GLO system, thereby disabling the brain's defenses against glycation stress imposed by methylglyoxal. GAPDH inactivation may have intensified methylglyoxal formation, and inactivation of GR would deprive the GLO system of its essential co-factor, GSH. Collectively, these impairments would leave the cerebral parenchyma vulnerable to the powerful glycating carbonyl, methylglyoxal. This study also demonstrated protection of GLO1, GR, and GAPDH by pyruvate, a heretofore unrecognized cerebroprotective action of the intermediary metabolite. By focusing on the methylglyoxal glycation pathway that exacerbates postischemic brain injury, this study identified pyruvate as a first intervention able to preserve antiglycation defenses in the brain, which may synergize with pyruvate's other cerebroprotective actions. The possibilities that pyruvate's acute antiglycation actions support sustained neurobehavioral recovery following CA, and that pyruvate may exert similar effects in traumatic brain injury, stroke, and other neurological disorders, merit investigation.

**Authors' contributions:** GFS conceived the study, conducted immunoblots, and performed data reduction and statistical analyses. AQN, BHC, RAH, AGW, and MGR performed surgical preparation, conducted CA/CCR experiments, cross-perfused the brain, and collected and analyzed plasma samples. GFS and MGR collected brain

samples, which were extracted by GFS and IS. GFS, RAH and IS conducted enzyme assays and statistical analyses. GFS and RTM evaluated the literature and drafted the manuscript. GFS, BHC, and AQN prepared the figures. All authors edited the manuscript.

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#### DECLARATION OF CONFLICTING INTERESTS

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