Neurobiology of Disease 45 (2012) 177–187

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

# Pyruvate incubation enhances glycogen stores and sustains neuronal function during subsequent glucose deprivation

Pavan K. Shetty *, Matthew P. Sadgrove, Francesca Galeffi, Dennis A. Turner

*Neurosurgery and Neurobiology, Duke University Medical Center, Research and Surgery Services, Durham VAMC, NC, 27710, USA*

ARTICLE INFO

*Article history:*
Received 31 March 2011
Revised 11 July 2011
Accepted 3 August 2011
Available online 10 August 2011

*Keywords:*
Glucose deprivation
Pyruvate
Glycogen
Hippocampus
Synaptic function

ABSTRACT

The use of energy substrates, such as lactate and pyruvate, has been shown to improve synaptic function when administered during glucose deprivation. In the present study, we investigated whether prolonged incubation with monocarboxylate (pyruvate or lactate) prior rather than during glucose deprivation can also sustain synaptic and metabolic function. Pyruvate pre-incubation(3–4 h) significantly prolonged (>25 min) the tolerance of rat hippocampal slices to delayed glucose deprivation compared to control and lactate pre-incubated slices, as revealed by field excitatory post synaptic potentials (fEPSPs); pre-incubation with pyruvate also reduced the marked decrease in NAD(P)H fluorescence resulting from glucose deprivation. Moreover, pyruvate exposure led to the enhancement of glycogen stores with time, compared to glucose alone (12 μmol/g tissue at 4 h vs. 3.5 μmol/g tissue). Prolonged resistance to glucose deprivation following exogenous pyruvate incubation was prevented by glycogenolysis inhibitors, suggesting that enhanced glycogen mediates the delay in synaptic activity failure. The application of an adenosine A1 receptor antagonist enhanced glycogen utilization and prolonged the time to synaptic failure, further confirming this hypothesis of the importance of glycogen. Moreover, tissue levels of ATP were also significantly maintained during glucose deprivation in pyruvate pretreated slices compared to control and lactate. In summary, these experiments indicate that pyruvate exposure prior to glucose deprivation significantly increased the energy buffering capacity of hippocampal slices, particularly by enhancing internal glycogen stores, delaying synaptic failure during glucose deprivation by maintaining ATP levels, and minimizing the decrease in the levels of NAD(P)H.



## Introduction

Mature brain depends on glucose as a primary metabolic substrate, which is either metabolized directly by neurons or metabolized to lactate in glia and exported to neurons as an energy source (Brown et al., 2003; Dringen et al., 1993; Pellerin et al., 1998; Wender et al., 2000). Brain can store glucose in the form of glycogen in astrocytes, which can serve as an immediate energy source when glucose supply is limited. However, brain normally has low glycogen levels (3–12 μmol glycosyl units/g tissue), insufficient to support neuronal function even for a short period of exposure to low glucose concentration (Cataldo and Broadwell, 1986; Choi and Gruetter, 2003; Cruz and Dienel, 2002; Dringen and Hamprecht, 1992; Sang Won et al., 2007; Wender et al., 2000). Although astrocytes have glucose 6-phosphatase activity (Ghosh et al., 2005), astroglia only extrude glucose (from glycogen breakdown) in some limited situations (Eyre et al., 1994; Walz and Mukerji, 1988). Neurons therefore primarily rely on astrocytic-derived lactate resulting from glycogen breakdown for energy support during glucose deprivation (Brown et al., 2005; Dringen et al., 1993; Pellerin and Magistretti, 1994; Pellerin et al., 2007; Sickmann et al., 2009; Walz and Mukerji, 1988). In addition to astrocyte-derived lactate brain can also utilize ketone bodies, lactate and pyruvate derived from systemic sources during hypoglycemia.

Although lactate can maintain neuronal synaptic function, pyruvate, another key intermediate in the metabolism of glucose, may be a better energy substrate after hypoglycemia than lactate in neurons (Suh et al., 2005). For example, depletion of cytoplasmic $NAD^+$ by poly (ADP-ribose) polymerase-1 (PARP-1) activation during hypoglycemia can render neurons unable to use glucose and lactate (Suh et al., 2005) whereas exogenous pyruvate can still be effectively metabolized to produce ATP. Supplementation with pyruvate can also support neuronal function, particularly in the absence of glucose (Maus et al., 1999). However, any protective effect of systemic pyruvate depends

*Abbreviations:* fEPSP, field excitatory postsynaptic potential; A1, adenosine receptor 1; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DAB, 1, 4-dideoxy-1,4-imino-D-arabinitol; NJM, 1-deoxyjirinomycin; ACSF, artificial cerebrospinal fluid; CA1, cornu ammonis region 1; DG, dentate gyrus; SR, stratum radiatum; AG, amyloglucosidase; glc, glucose; pyr, pyruvate; con, control; GIs, glycogenolysis inhibitors; ROI, region of interest; MCT, monocarboxylate transporter.

* Corresponding author at: Box 3807, Neurosurgery, DUMC, Durham, NC 27710, USA. Fax: +1 919 681 8068.
*E-mail address:* shetty.p@duke.edu (P.K. Shetty).

**Available online on ScienceDirect (www.sciencedirect.com).**

0969-9961/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2011.08.002

upon the properties of its transport across the blood brain barrier. The blood brain barrier in adults normally transports pyruvate and lactate at a slower rate than glucose, due to limited expression of monocarboxylate transporters, but pyruvate entry to the brain can be achieved by elevating the plasma pyruvate concentration (Lee et al., 2001; Oldendorf, 1973; Suh et al., 2005).

Utilization of either glucose or monocarboxylates can demonstrate advantages and disadvantages as energy sources under certain conditions. Lactate and pyruvate are preferred over glucose when ATP levels are limited because utilization of glucose requires ATP-driven phosphorylation of glucose, whereas pyruvate and lactate can be oxidatively metabolized directly. Thus, both lactate and pyruvate can protect hippocampal neuronal function during glucose deprivation (Takata et al., 2001). Likewise, post-hypoglycemic pyruvate treatment in vivo also decreased cell death in rats (Suh et al., 2005), possibly by directly enhancing mitochondrial metabolism.

The beneficial effects of either lactate or pyruvate on neuronal function have been focused on administration either during or after hypoglycemia; however, it remains unclear whether providing exogenous pyruvate and lactate solely during a period prior to glucose deprivation can also maintain neuronal function. In the present study, we investigate the effect of prior incubation of pyruvate and lactate on neuronal function during delayed glucose deprivation by measuring neuronal synaptic activity, NAD(P)H fluorescence and levels, glycogen and ATP levels.

## Methods

All animal use (5–6 weeks old Fischer 344 rats, Harlan, Indianapolis, IN, USA) was approved by the Animal Care and Use Committees of both Durham VAMC and Duke University Medical Center. Other materials used in the present study were obtained from the indicated sources: sodium pyruvate, bovine serum albumin, glucose assay kit, amyloglucosidase (AG), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), 1-deoxyjirinomycin (NJM) (Sigma Aldrich), Bradford protein assay reagent (Bio-Rad), ATP assay kit, $NAD^+$/NADH assay kit (Abcam).

### *Hippocampal slice preparation*

Transverse hippocampal slices (400 μm) were prepared from male rats as previously described (Galeffi et al., 2007; Sadgrove et al., 2007) and were kept in oxygenated (95% $O_2$/5% $CO_2$) ACSF (in mM: NaCl 124, KCl 3.0, $NaH_2PO_4$ 1.25, $NaHCO_3$ 24, $CaCl_2$ 2.0, $MgSO_4$ 2.0, and dextrose 10, pH 7.4) at room temperature (22 °C) for 1 h for recovery. The slices were then transferred to oxygenated ACSF with and without 10 mM pyruvate (with changes in NaCl concentration to maintain osmolality) and incubated at 32 °C for 2–6 h.

### *Synaptic stimulation*

Hippocampal slices were placed in an interface recording chamber maintained at 36º C and an insulated stainless steel bipolar stimulating electrode placed in stratum radiatum to stimulate Schaffer collateral/commissural fibers. Field excitatory post synaptic potentials (fEPSPs) were recorded using glass microelectrodes filled with 1 M NaCl, placed in the stratum radiatum of the CA1 region. Slice were perfused with ACSF at a flow rate of 2 ml/min and test pulses were delivered at 30-s intervals with a constant current stimulus (0.1 ms, 0.1 to 0.3 Hz, up to 0.2 mA), evoking fEPSP responses equivalent to 50% of the maximum response. Both the amplitude and the slope of the negative fEPSP evoked response were measured and the % changes were similar during experimental manipulations; our data show only fEPSP amplitude. After recording a stable baseline for at least 10 min, slices were subjected to different experimental conditions. Glucose deprivation was induced by modified glucose-free ACSF with the addition of 10 mM sucrose to maintain physiological osmotic pressure and no monocarboxylates were present in this perfusion solution. The effects of glycogenolysis inhibitors (GIs) (100 μM DAB—a glycogen phosphorylase inhibitor, and 100 μM NJM—a α-glucosidase inhibitor) and an adenosine A1 receptor antagonist (1 μM DPCPX) were tested during glucose deprivation after acquiring a stable baseline with regular ACSF for least 10 min.

### *NAD(P)H imaging*

NAD(P)H is fluorescent in its reduced state but not in its oxidized state ($NAD(P)^+$); hence a change in NAD(P)H fluorescence is a sensitive indicator of cellular redox state and rate of oxidative energy metabolism. Changes in NAD(P)H fluorescence in hippocampal slices were monitored using a 290–370 nm excitation filter and a 420 nm emission filter (xenon light source, DG-4, Sutter), as described previously (Foster et al., 2008). In brief, the slices in the interface chamber were imaged through a Nikon upright microscope (UM-2) with a compound lens (4×, N.A 0.13) using a linear, cooled 12 bit CCD camera with 1280×1024 digital spatial resolution (Sensicam QE). Images were acquired every 5 s with ~0.5 s exposure for each image. The imaging analysis consisted of creating a region of interest (ROI) within stratum radiatum (SR), between the stimulating and recording electrodes, and then calculating the average number of pixels within each region of interest for each image. The net difference across the image series was then calculated as $\Delta F/F = 100 \times \{(\text{ROI: image} - \text{ROI: baseline})/\text{ROI: baseline}\}$.

### *Glycogen measurements*

Hippocampal slices were removed (during pre-incubation and glucose deprivation studies) from the medium at different time intervals and immediately placed in ice cold 85% ethanol containing 15% of 30 mM HCl to arrest both glycogenesis or glycogenolysis followed by storage in liquid $N_2$. Frozen slices were dried on Whatman #1 filter paper and homogenized in a volume (110 μl/3 slices) of 0.1 M NaOH with 0.01% SDS and 1 mM EDTA, using a sonicator. Homogenates were neutralized with 0.3 N HCl (32 μl). Then, 50 μl of paired homogenates were added to 250 μl of 50 mM sodium acetate buffer (pH 5.5) for glycogen hydrolysis, with and without 1 unit of amyloglucosidase (AG) to measure total and free glucose levels, respectively. Samples were incubated at 32 °C for 2 h then cooled on ice and then centrifuged at 14,000 rpm for 20 min. 100 μl of the supernatant from the digested samples was used for glucose measurements (Swanson et al., 1989). The free and total glucose contents were measured by a glucose assay kit according to the manufacturer instructions (Sigma Aldrich). In brief, glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of oxidized nicotinamide adenine dinucleotide ($NAD^+$) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of $NAD^+$ is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to glucose concentration. The protein concentrations of the homogenates were estimated by Bradford dye binding method to normalize the glycogen contents (nmol) per mg protein. A standard glucose plot was established to calculate the glycogen content, using a conversion factor of 180 g/mol free glucose = 162 g/mol glycosyl units of glycogen (Abdelmalik et al., 2007). The glycogen units are presented as μmol glycosyl unit/g wet tissue weight by considering the fact that protein content in the brain constitutes 11.7% of wet brain weight (Choi and Gruetter, 2003).