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INCREASED GLUCOSE AVAILABILITY DOES NOT RESTORE PROLONGED SPREADING DEPRESSION DURATIONS IN HYPOTENSIVE RATS WITHOUT BRAIN INJURY

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Abstract

Maintenance of transmembrane ionic gradients and their restoration after cortical spreading depression (CSD) are energy dependent. We recently showed an inverse relationship between blood pressure and CSD duration that is independent of tissue oxygenation. Here, we tested the alternative hypothesis that glucose availability becomes rate-limiting for CSD recovery upon reduced blood pressure in anesthetized rats under full systemic physiological monitoring. Hypotension induced by controlled exsanguination significantly prolonged CSD durations, reduced propagation speeds, and diminished the blood flow response. Hyperglycemia failed to restore the prolonged CSD durations in hypotensive rats and did not significantly alter the propagation speed or the blood flow response. These data suggest that prolonged CSD durations during reduced cerebral perfusion pressure are independent of tissue energy status, and implicate alternative mechanisms of CSD recovery such as vascular clearance of extracellular K⁺.

Keywords

Hyperglycemia; hypotension; spreading depression

INTRODUCTION

Cortical spreading depression (CSD) is associated with a marked surge in O₂ and glucose utilization in both normal and ischemic brain. Indeed, severe hypoxia or hypoglycemia can delay the recovery of CSD, and even precipitate persistent depolarization, indicating that maintenance and restoration of membrane ionic gradients are dependent on cellular energy

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status (Gido *et al* 1993). Because of this, the prolonged durations of peri-infarct depolarization (PID) waves in ischemic penumbra have been explained by O₂ and glucose shortage. This was further supported by the observation that PIDs become indistinguishable from CSD waves as they propagate into the surrounding non-ischemic brain.

Consistent with this notion, we recently showed that CSD duration is inversely related to cerebral perfusion pressure in rats, so that systemic hypotension significantly prolonged the slow potential shift and delayed CSD recovery (Sukhotinsky *et al* 2008; Sukhotinsky *et al* 2010). However, systemic hyperoxia did not restore prolonged CSD durations in hypotensive rats despite restored tissue pO₂, suggesting that the inverse relationship between cerebral perfusion pressure and CSD duration is independent of cortical O₂ availability and oxidative phosphorylation (Sukhotinsky *et al* 2010). Because glycolysis is rapidly stimulated during CSD (Csiba *et al* 1985; Hashemi *et al* 2009; Shinohara *et al* 1979), glucose may be a more relevant and immediate energy source impacting restoration of membrane ionic gradients, and may become rate-limiting during CSD. We, therefore, tested whether prolonged CSD durations in hypotensive rats can be reversed by increasing tissue glucose availability.

METHODS

General surgical preparation

National and institutional guidelines for animal care and use for research purposes were strictly followed, and study protocol was approved by the institutional review board. Rats (Sprague-Dawley, male, 275–400 g) were fasted overnight, and anesthetized with urethane (1.3–1.5 g/kg, intraperitoneal), and intubated for mechanical ventilation (70% N₂O/30% O₂ during preparation, room air during the experiment; SAR-830, CWE, PA, USA). Femoral vein and artery were cannulated for infusions, continuous mean arterial blood pressure (MABP) recording (PowerLab, ADInstruments, MA, USA), and arterial blood gas and pH measurements every 15–30 minutes to maintain arterial pCO₂ (p_aCO₂) around 35 mmHg (Rapidlab 248 blood gas/pH analyzer, Bayer HealthCare). Blood glucose was measured before each CSD, and cerebrospinal fluid (CSF) glucose at the end of each experiment via cisternal puncture. Rectal temperature was kept at 37°C using a thermostatically controlled heating pad (Harvard Apparatus, MA, USA). Rats were placed in a stereotaxic frame (Stoelting Co, IL, USA) and 3 burr holes were drilled under saline cooling over the right hemisphere at the following coordinates (mm from bregma): (i) posterior 6, lateral 3 (occipital cortex) for topical KCl application; (ii) posterior 2, lateral 3 (parietal cortex) for electrophysiology and laser Doppler flowmetry; (iii) anterior 2, lateral 3 mm (frontal cortex) for electrophysiology. Dura was gently removed at the KCl and laser Doppler sites, and care was taken to avoid bleeding. Following surgical preparation, the cortex was allowed to rest for 10 minutes under saline irrigation and dura covered with mineral oil to prevent drying.

Electrophysiology

The extracellular slow (DC) potential changes and electrocorticogram (ECoG) were recorded with glass micropipettes (150 mM NaCl) 300 µm below dura (EX1 differential amplifier, Dagan Corporation, MN, USA). Ag/AgCl reference electrode was placed subcutaneously in the neck. The DC shift amplitude and its duration at half maximal amplitude were measured. SD propagation speed was calculated based on its latency and the distance between the two recording electrodes.

Cerebral blood flow (CBF)

Regional CBF was recorded using laser Doppler flowmetry (LDF) (ADInstruments, CO, USA). The LDF probe (0.48 mm tip diameter) was placed in the immediate vicinity of

proximal glass micropipette away from large vessels. The amplitudes and latencies of CBF changes were measured at baseline (time 0), peak, recovery, 5 and 8 minutes after CSD onset, expressed as % of initial baseline prior to the onset of the experimental protocol, and plotted as a function of time.

Experimental protocol

After stable baseline electrophysiological and LDF recordings, a CSD was induced under normotensive normoglycemic conditions. Arterial blood pressure was then reduced to 40–45 mmHg by controlled exsanguination, and a second CSD was triggered. Intravenous 20% dextrose (hyperglycemia group) or saline (vehicle control) infusion was then started and continued for 1 hour; saline infusion served as time control. Hyperglycemia group also received an additional dose (1–2ml) of 40% dextrose intraperitoneally. After confirming target blood glucose (approximately 400 mg/dL in hyperglycemia group), a third CSD was induced.

RESULTS

Systemic physiological parameters were within previously reported normal limits in normotensive, normoglycemic, anesthetized rats (Figure 1). Hypotension by controlled exsanguination was associated with mild metabolic acidosis and reduction of p_aO_2 within physiological limits, neither of which were altered by subsequent hyperglycemia. Blood glucose showed a small but steady increase during the course of each experiment, presumably related to anesthesia and surgery. Dextrose infusion raised blood glucose by more than two-fold and CSF glucose by 75% compared to saline-infused time controls (Figure 1).

Systemic hypotension (blood pressure 40–45 mmHg) prolonged the CSD duration by approximately 40–70% and reduced propagation speed by 20–25% without altering its amplitude (not shown), as reported previously (Sukhotinsky *et al* 2010). Hyperglycemia failed to normalize the CSD duration and the propagation speed in hypotensive rats (Figure 1).

In normotensive, normoglycemic rats CSDs were associated with a more than 2-fold increase in CBF lasting approximately 2 minutes (Figure 2). Hypotension decreased resting CBF by 25–30% ($p>0.05$), significantly diminished and prolonged the hyperemic response to CSD, and brought out an initial hypoperfusion preceding the hyperemia ($12\pm6\%$), as reported previously (Sukhotinsky *et al* 2008). Hyperglycemia did not alter the initial hypoperfusion during CSD ($12\pm6\%$) or the peak hyperemic response (Figure 2), consistent with previous reports (Wolf *et al* 1997).

DISCUSSION

We showed that, despite a significant increase in tissue glucose availability, hyperglycemia does not restore the delayed CSD recovery during hypotension. Taken together with our previous demonstration that increasing O_2 availability by normobaric hyperoxia is also ineffective (Sukhotinsky *et al* 2008; Sukhotinsky *et al* 2010), and that the magnitude of hypotension sufficient to prolong CSDs causes only mild hypoperfusion (i.e., 10–30% decrease in resting CBF), these data do not support the notion that prolonged CSDs during reduced perfusion pressure are due to energy shortage. It should be noted that plasma glucose does impact the duration of spontaneous CSDs in the more severely hypoperfused penumbra, as well as the latency to develop anoxic depolarization (Bures *et al* 1974; Hansen 1978; Nedergaard and Astrup 1986). Therefore, our data should not diminish the importance of plasma glucose management for the outcome of injured brain (Strong 2008).

Arguably, the most critical factor governing the repolarization rate during CSD is restoration of extracellular K^+ ($[K^+]_e$) from tens of millimolars to the physiological range of a few millimolars. Primary regulators of $[K^+]_e$ are the Na^+/K^+ -ATPase and the astrocytic spatial buffering. Because shortage of tissue energy substrates (i.e., oxygen and glucose) does not appear to contribute, it is unlikely that inhibition of Na^+/K^+ -ATPase is an important mechanism for CSD prolongation when perfusion pressure is reduced. Astrocytic spatial buffering is important for maintaining $[K^+]_e$ during physiological neuronal activity; however, its capacity is likely limited when large swaths of brain tissue (a few millimeters) is simultaneously bathed in massive levels of $[K^+]_e$. It is also unlikely that reduced perfusion pressure somehow interferes with astrocytic spatial buffering.

An alternative hypothesis that is independent of energy metabolism but still linked to tissue perfusion is vascular clearance of $[K^+]_e$. While small physiological rises in $[K^+]_e$ (<10 mM) during normal brain activity are rapidly cleared by local neurons and astrocytes, the massive $[K^+]_e$ increase during CSD (30–80 mM) can readily flood the perivascular space either via passive diffusion or through astrocyte end-feet, and cleared into the microvasculature by active or facilitated transport. Such systems are indeed present on the abluminal membranes of cerebrovascular endothelium (Bradbury *et al* 1972; Goldstein 1979). In support of this, a net loss of total brain tissue K^+ has been demonstrated after anoxic depolarization with permanent $[K^+]_e$ elevations comparable to CSD (Hu *et al* 2000; Yushmanov *et al* 2011). Although vascular clearance is an attractive hypothesis to explain delayed CSD recovery during reduced cerebral perfusion pressure, direct evidence in support of such a mechanism is still lacking.

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Figure 1. Hyperglycemia does not restore altered electrophysiological attributes of cortical spreading depression in hypotensive rats

Representative electrophysiological tracings from the same rat show prolonged spreading depression during hypotension was not restored by superimposed hyperglycemia. Graphs summarize spreading depression duration and speed from vehicle and hyperglycemia groups at different stages of the experiment. Systemic physiological parameters at each successive stage are shown at the bottom. In a separate normotensive control cohort, CSF glucose was 98 ± 10 mg/dL ($n=10$); therefore, hypotension *per se* did not diminish cerebral glucose availability. BP, blood pressure (mmHg); BG, blood glucose (mg/dL); CSFG, cerebrospinal fluid glucose (mg/dL); NT, normotension; NG, normoglycemia; HT, hypotension; HG, hyperglycemia. ^a $p < 0.05$ vs. NT/NG; ^b $p < 0.05$ vs. all other groups; ^c $p < 0.05$ vs. HT/NG2; ^d $p < 0.05$ vs. HT/NG1; ^e $p < 0.05$ vs. HT/NG. Mean $\pm$ SEM.

Figure 2. Hyperglycemia does not alter the hemodynamic response to cortical spreading depression in hypotensive rats

Graphs summarize cortical spreading depression-induced cerebral blood flow changes during successive stages of the experimental protocol. Hypotension diminished the magnitude of the large hyperemic response to spreading depression ($p < 0.05$), and delayed its peak and recovery ($p < 0.05$), when compared to the normotensive normoglycemic baseline response. Hyperglycemia did not restore these changes to normotensive normoglycemic baseline values. We used a mixed model analysis of covariance for repeated measures with blood glucose and blood pressure as fixed effects to compare mean values over experimental stages (I: normotensive normoglycemic baseline, II: hypotensive normoglycemic stage, III: hypotensive hyperglycemia or vehicle time control), and according to the group (normoglycemia vs. hyperglycemia). Vertical and horizontal error bars indicate the standard error of the magnitude and latency of deflection points from the onset of hyperemia.