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Research Report

Effects of glucose and glutamine concentration in the formulation of the artificial cerebrospinal fluid (ACSF)

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ABSTRACT

The composition of the ACSF is fundamental in controlling the extracellular environment of the brain slice preparation. 'Typical' formulations lack amino acids and contain a higher concentration of glucose (10 mM) than in the cerebrospinal fluid (0.47 – 4.4 mM). We examined the effects of different concentrations of glutamine, the most abundant amino acid in the CSF, and glucose on rat hippocampal slice physiology. Bipolar paired-pulse stimulation was applied to the Schaffer collaterals and population spikes were monitored in the CA1 pyramidal layer for ~1 hour. Addition of glutamine (0.5 mM) to slices superfused with 10 mM of glucose did not enhance population spike amplitude. Higher concentration of glutamine (2-5 mM) resulted in spreading-depression. Decreasing glucose concentration from 10 mM to 5 mM, in the absence of glutamine, attenuated population spikes. Decreasing glucose to 2 mM, in the absence of glutamine, suppressed evoked population spikes. Superfusing brain slices with ACSF containing 'physiological' concentrations of both glucose (2 mM) and glutamine (0.5 mM) similarly suppressed population spikes. In separate experiments, during high-K^+ induced epileptiform activity, glutamine (0.5 mM) did not affect the burst duration, frequency or waveform. These results suggest that the concentration of glucose in ACSF should conservatively be 10 mM in order to maximize paired-pulse population responses while the presence of physiological concentration of glutamine (0.5 mM) has minimal effects on paired-pulse responses and high-K^+ induced epileptiform activity. These results are discussed in the context of fundamental differences between *in vitro* brain slice superfusion and *in vivo* brain perfusion.

1. Introduction

The composition of artificial cerebrospinal fluid (ACSF) is fundamental in controlling the extracellular environment of the *in vitro* brain slice preparation and will affect the 'baseline' levels of neuronal activity (e.g. resting membrane potential, action potential rate, transporter activity, intracellular ion content), metabolism (e.g. energy-related parameters, oxygen and glucose consumption), and protein synthesis (Jefferys, 1995). Despite the ubiquity and established utility of the brain slice preparation, there are significant variations in ACSF composition across discrete slice studies and there remain fundamental questions about 'appropriate' ACSF formulation; moreover, typical ACSF formulation deviate fundamentally from *in vivo* brain organic concentrations.

In the brain, glucose is a key energy substrate due to its high plasma concentration and abundant glucose transporters across the blood-brain-barrier (Dienel and Hertz, 2001). In

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'typical' ACSF formulations, glucose concentration is 10-11 mM (in some studies up to 25 mM; Christie and Jahr, 2006). These values are significantly higher than the CSF glucose range reported, 0.47-4.4 mM (Silver and Erecinska, 1994; McNay and Gold, 1999; Kirchner et al., 2006). *In vitro*, the minimum glucose concentration required to maximize *specific* electrophysiological indicators, including population spikes, has been reported to vary in the range of 1 to 10 mM (Cox and Bachelard, 1982; Schurr et al., 1989; Rosen and Andrew 1991; Yuan et al., 2004; Kirchner et al., 2006). Increasing glucose concentration (>5 mM) exerts a neuroprotective role *in vitro* (Schurr et al., 1989; Schurr et al., 1999a,b; Cater et al., 2003). The 'appropriate' glucose concentration in ACSF thus remains controversial.

In addition to glucose, the cerebrospinal fluid (CSF) contains numerous amino acids of which glutamine is the most abundant, in the range of 0.4-0.8 mM (Ames and Nesbett, 1981; Schiff et al., 1985; Szerb and O'Regan, 1985; Schurr et al., 1987; Kapetanovic et al., 1993; Nishimura et al., 1995; Zheng et al., 2000, Tani et al., 2007). Glutamine serves as a major precursor of neurotransmitters including glutamate and GABA (Szerb and O'Regan 1985; Battaglioli and Martin 1991; Berg-Johnsen et al., 1993; Bacci et al., 2002; Bak et al., 2006; Kam and Nicoll 2007; Tani et al., 2007), has been found to induce enzyme activity (Baudry et al., 1988) and may serve as energy substrate (Bradford et al., 1978; Bacci et al., 2002; Cater et al., 2003). However, glutamine is omitted in 'typical' ACSF formulations. Superfusion with glutamine-free ACSF leads to depletion of brain slice glutamine content (and related neurotransmitter content), which can be (partially) reversed by addition of glutamine to the ACSF (Kapetanovic et al., 1993; Battaglioli and Martin, 1991; Tani et al., 2007). Experiments with fluorcitrate suggest that inhibition of glutamine synthesis disrupts brain slice function (Berg-Johnsen et al., 1993). The effects of ACSF glutamine on slice physiology appear system and experimental protocol specific (Schiff et al., 1985; Kam and Nicoll 2007; Tani et al., 2007).

To better understand the role of glucose and glutamine in ACSF formulation, we examined the effects of glutamine and different concentrations of glucose on evoked paired population responses in rat hippocampal slices and spontaneous epileptiform activity. The goal of this study was to determine what concentrations of ACSF glucose and glutamine are required to support slice function; paired-population responses and epileptiform activity are sensitive to a broad range of excitability and excitatory/inhibitory synaptic changes. In addition to extending previous work examining the effects of ASCF glutamine (in the presence of physiologically 'high' glucose concentration) or only reducing ACSF glucose concentration (in the absence of glutamine), we specially investigated the concurrent restoration of *both* ACSF glucose and glutamine to *in vivo* levels.

As a result of physical slicing, inherent connectivity/morphological, metabolic, and neurophysiological differences exist between *in vivo* and *in vitro* brain tissue (Elliot 1969; Dingledine et al., 1980; Langmoen and Andersen 1981; Lipton and Whittingham 1984; Schwartzkroin et al., 1998; Schurr et al., 1999b; Zheng et al., 2000; Novotny et al., 2001). There are fundamental differences between brain slice superfusion and brain perfusion, including blood, CSF, and interstitial flow (Fig. 1). Because of these inherent differences, ACSF formulations closer to CSF concentrations may not necessarily result in more 'relevant' *in vitro* model systems (Kirchner et al 2006). In general, the relevance of data collected using brain slices to the *in vivo* situation is dependent on how well 'important' *in vivo* characteristics are preserved *in vitro*; which *in vivo* characteristics are considered important depends on the questions asked (Alger et al., 1984). The conditions of brain slice preparation and maintenance, including the formulation of the slice superfusate (ACSF), are thus critical to the valid interpretation of *in vitro* data. A necessary first step toward addressing this complex topic is to quantify what effects compounds normally present in CSF, including glucose and glutamine, have on brain slice function.

2. Results

2.1. *Effect of ACSF glutamine and glucose concentration of evoked population spikes*

Paired-pulse evoked population spikes provide a gross indicator of overall slice excitability, including synaptic function.

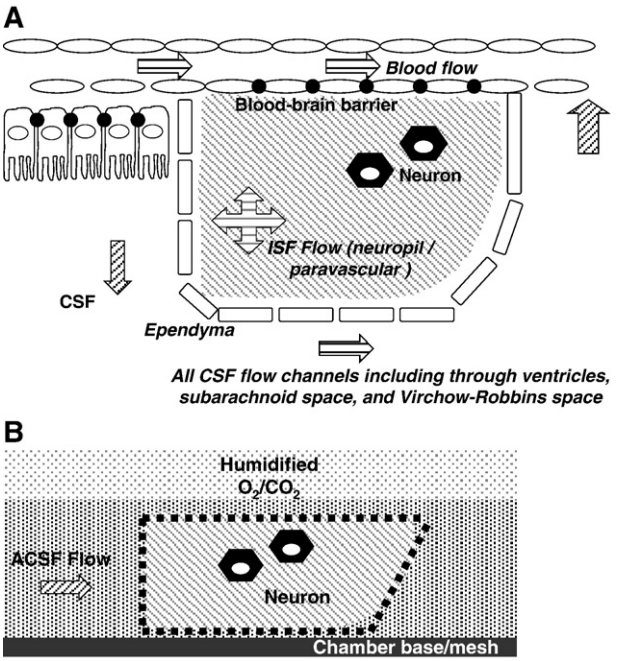

Fig. 1 – Schematic of key A) *in vivo* brain perfusion mechanisms and B) *in vitro* superfusion mechanisms relating to metabolite/reactant delivery/clearance. Mechanical slicing of tissue results in 'fundamental' traumas regardless of the details of the preparation method. During slice preparation and handling some mechanical damage occurs at the boundary of the slice (dashed line). The vasculature of the slice is rendered inoperative. In the absence of circulation, blood vessels no longer move substances such as oxygen and glucose into the tissue and CO_2 out of the tissue. In addition, CSF flow and bulk flow of interstitial fluid no longer operate as a transport mechanism for substances into and through the brain. Surface ACSF flow is the only convective mechanism for transport remaining in the slice. In brain slices, the delivery and clearance kinetics of metabolites is limited by diffusion with the surface.

The role of glutamine in ACSF was tested by monitoring its effect on population spike amplitude. Under both control (10 mM glucose, 0 mM glutamine) and glutamine (10 mM glucose, 0.5 mM glutamine) conditions, population spike amplitude increased gradually over the course of most experiments. In this and other tests, the role of over time was therefore compared to control drift in population spike amplitude. There was no difference in normalized population spike amplitude at 50 minutes, between control (n=7) and glutamine-treated (n=18; 0.5 mM) slices (control P1, 192±34%; glutamine-treated P1, 155±14%; control P2, 127±11%; glutamine-treated P2, 160±83%; Fig. 2B). There was no significant difference in paired-pulse ratio for either facilitation or depression cases (control PPF, 85±20%; glutamine-treated PPF, 99±8%; control PPD, 59±11%; glutamine-treated PPD, 120±33%; Fig. 2C). Lower concentration of glutamine (0.2 mM) similarly had no effect on population spike amplitude (n=3; not shown). Higher concentration of glutamine (2-5 mM) induced spreading-depression in 5 of 6 slices tested (Fig. 3), in the remaining slice the population spike amplitude attenuated over time.

The concentration of glucose in ACSF was varied while monitoring evoked population spike amplitude. Reduction of glucose from 10 mM to 5 mM (n=6) resulted in a significant decrease in the pulse 1, but not pulse 2, population spike amplitude with respect to control cases (control P1, 192±34%; 5 mM glucose-treated P1, 92±21%; control P2, 127±11%; 5 mM glucose-treated P2, 113±28%; Fig. 4B). There was no significant difference in paired-pulse ratio for either facilitation or depression cases (control PPF, 85±20%; 5 mM glucose-treated PPF, 113±72%; control PPD, 59±11%; 5 mM glucose-treated PPD, 126±25%; Fig. 4C). Reduction of glucose to 2 mM significantly attenuated P1 population spike amplitude such that within 30 minutes of low-glucose expose, P1 spikes were reduced to 41±21% of baseline, while at 30 minutes P2 population amplitude was not reduced significantly (78±28%). At 50 minutes, both pulses were significantly attenuated relative to

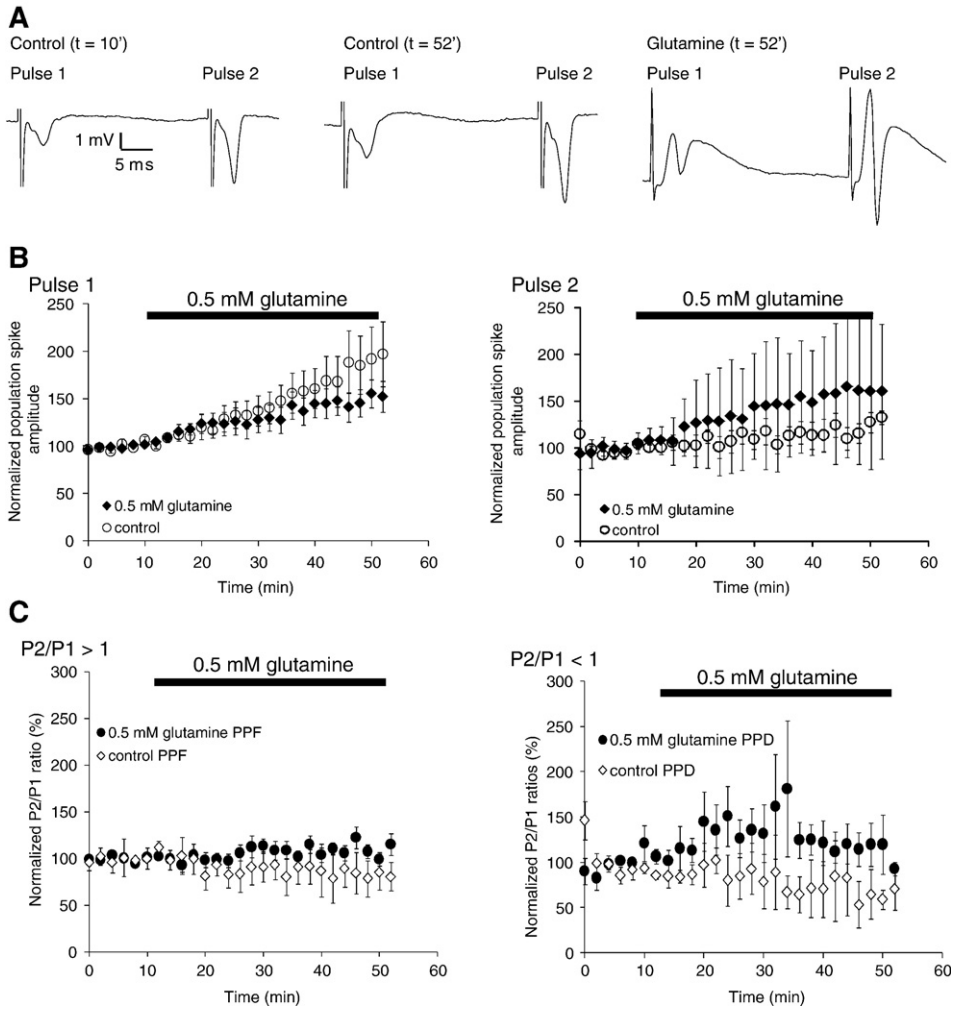

Fig. 2 – Effect of 0.5 mM glutamine on orthodromic population spikes evoked in the CA1 region of hippocampal brain slices. A) Sample traces of control and glutamine- treated population spikes. B) Paired-pulses were delivered to the Schaeffer-collaterals every 2 minutes and supra-threshold responses monitored in the CA1 pyramidal layer. Extracellular glucose was maintained at 10 mM. There was no significant difference between control (○; n=7) and 0.5 mM glutamine (♦; n=18) treated slices. C) No significant difference between control and glutamine-treated ratios for either facilitation or depression cases.

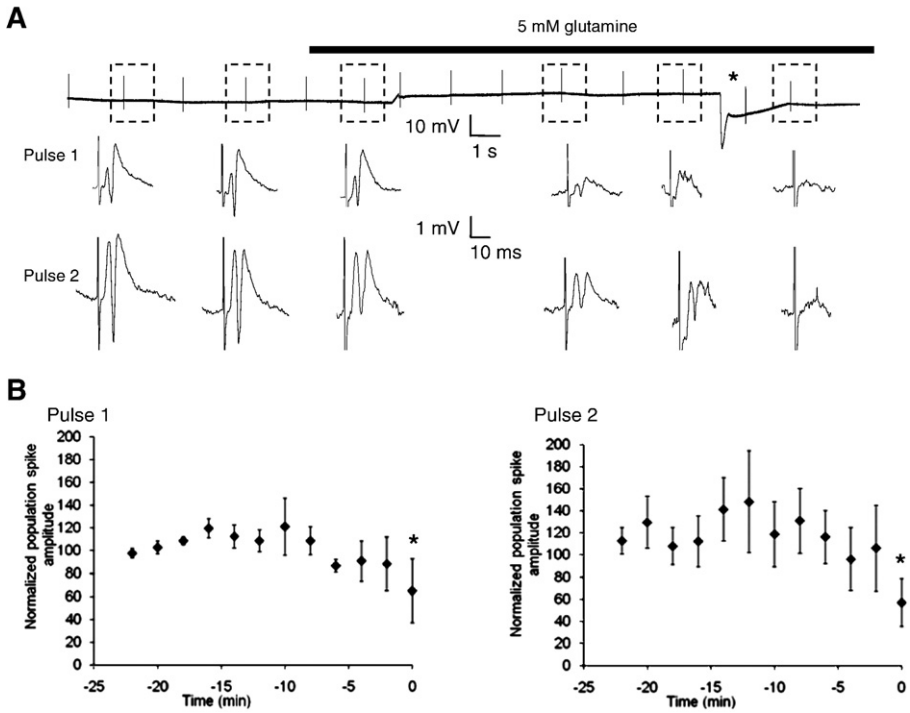

Fig. 3 – Effect of 2-5 mM glutamine on hippocampal brain slice neurophysiology. Baseline ACSF glucose was 5 mM. Asterisks (*) indicates initiation of spreading-depression. A) Continuous recording of CA1 field potential during application of 5 mM glutamine (bar). Paired-pulse orthodromic responses were monitored every 2 minutes. B) Average responses across slices; for each slice data is presented as time before of spreading-depression (at t = 0).

control conditions (2 mM glucose-treated P1, 34±23%; 2 mM glucose-treated P2, 62±26 %; Fig. 5B). These results are consistent with previous studies (Fan et al., 1988; Schurr, 1989) using single and paired-pulses where 'synaptic silence' was reported when glucose concentration was reduced below 2 mM.

After confirming that 2 mM glucose, in the absence of glutamine, is insufficient to maintain slice function, we investigated if the combination of 2 mM glucose and 0.5 mM glutamine could maintain or restore slice function. In this case, *both* glucose and glutamine were concurrently resorted to representative *in vivo* levels. We found that in the presence of glutamine (n = 5, 0.5 mM), reduction of glucose from 10 to 2 mM still caused disruption in the population response after 40 min exposure (P1, 134±23% to 39±16%; P2, 158±27% to 69±28%; Fig. 5C). These results indicate that glutamine (0.5 mM) cannot reverse the disruption in gross slice function resulting from glucose reduction to 2 mM.

We further tested if 'moderate' reductions in ACSF glucose (to 5 mM) would induce a (metabolic) dependence on ACSF glutamine. In the presence of baseline 5 mM glucose, addition of 1 mM glutamine had no significant effect of paired-pulse evoked orthodromic responses (not shown); spreading-depression was not observed in any slices after addition of 1 mM glutamine.

2.2. *Effect of ACSF glutamine on high-K^+ induced epileptiform activity*

We next investigated the effect of glutamine in high-K^+ ACSF, on slice function including cell excitability or synaptic function. Effects of ACSF glutamine on excitability of synaptic function would be expected to induce a change in high- K^+ epileptiform waveform (Jensen and Yaari, 1988; Leschinger et al., 1993; Bikson et al., 2002). In addition, we hypothesized that the increased neuronal activity/metabolic demand associated with epileptiform activity may induce a dependence on ACSF glutamine. However, addition of glutamine (0.5 mM; n = 4) did not affect high-K^+ induced epileptiform activity burst duration (116±9% of control), burst amplitude (114±11% of control), or burst frequency (89±8% of control; Fig. 6).

3. Discussion

Despite the acceptance of the brain slice as a valuable neuroscience research tool, there are significant differences in the ACSF formulation used across laboratories, and little consensus on 'appropriate' formulations, particularly concerning organics. The implications of the results of the present study, to this complex problem, are discussed.

3.1. *Role of ACSF glutamine*

Our experiments indicate that addition of glutamine to ACSF formulation does not significantly affect neuronal excitability or synaptic function (as reflected in evoked population spike response) during low-frequency stimulation of hippocampal brain slices over ~ 1 hour of recording time. Considering that *in vivo* clearance of glutamine from brain to endothelium or from CSF to choroids plexus is more rapid than the influx from blood

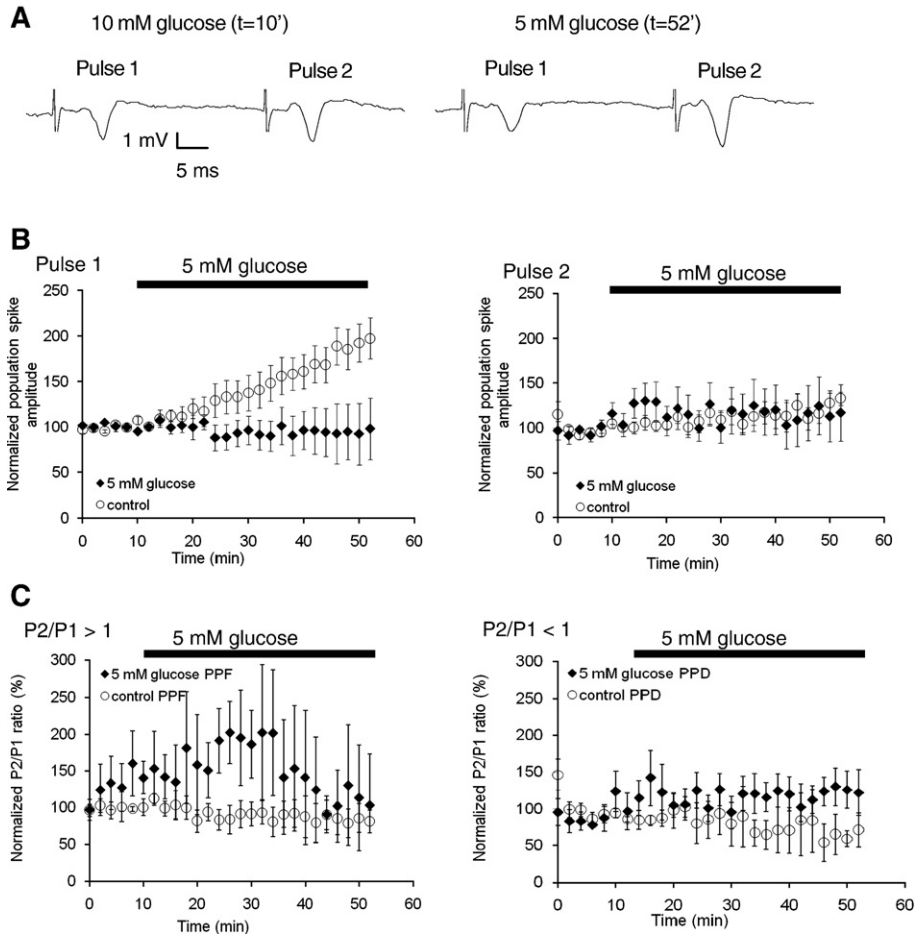

Fig. 4 – Effect of 5 mM glucose on orthodromic population spikes evoked in the CA1 region of hippocampal brain slices. A) Sample traces of superfusate population spikes 2 min before and 40 min after 5 mM glucose treatment. B) Extracellular glucose was reduced from 10 to 5 mM and paired-pulse responses monitored every 2 minutes. There was a significant difference between control (○; n = 7) and 5 mM glucose (♦; n = 6) treated slices only in pulse 1. C) There was no significant difference in paired-pulse ratio for either facilitation or depression cases.

(Xiang et al., 2003), our results strongly suggest a *net* production of glutamine by the brain (tissue) and efflux to the blood/ACSF. Under normal conditions, sufficient glutamine may be produced by brain slices (Berg-Johnsen et al., 1993), and excess glutamine diffused by the ACSF. Kapetanovic et al., (1993) reported time-dependent loss of glutamine in unstimulated slices. Thus, even in the absence of ACSF glutamine, brain slice neuronal glutamine pools may be adequately replenished, at least under the conditions tested in the present study. Considering that 0.5 mM ACSF glutamine results in overshoot of basal glutamine level (Kapetanovic et al., 1993), 'pathological' increases in ACSF glutamine concentration (2-5 mM) may *reverse* the direction of glutamine diffusion resulting in excessive interstitial glutamine accumulation, interference with glutamine-glutamate cycling, and/or saturation of amino acid transporters, ultimately leading to the spreading-depression observed in the present study and previously in cortical slices (Tani et al., 2007). Spreading-depression was not noted during 4 mM glutamine superfusion at room temperature (Kam and Nicoll 2007).

The glutamate-glutamine cycle is considered integral to replenishing the neurotransmitter pool (Pfrieger and Barres 1996; Tsacopoulos and Magistretti 1996; Rothman et al., 1999; Bacci et al., 1999; Bacci et al., 2002; Kam and Nicoll, 2007); recent reports have highlighted remaining questions regarding the kinetics of glutamine regulation (Winkler et al., 1999; Blin et al., 2002; Hasegawa et al., 2006; Masson et al., 2006; Conti and Melone, 2006; Kam and Nicoll 2007). Brain slice studies examining glutamine regulation have typically used relatively 'high' baseline glucose levels (compared to *in vivo* levels). Moreover, because of fundamental differences between slice and brain perfusion (Fig. 1), and related differences in metabolic kinetics, insights from *in vitro* studies must be cautiously applied to the *in vivo* situation. Rather, the focus of the present report was characterizing under what conditions ACSF glutamine would modulate the activity of brain slices and hence play a role in *in vitro* experimental design and interpretation.

The results of the present study indicate that omission of glutamine from ACSF formulation will not *necessarily* confound interpretation of results from brain slice studies. However, under conditions of prolonged (>2 hours) incubation or during intense neuronal activity, glutamine pools in the slice may be depleted resulting in functional uptake of

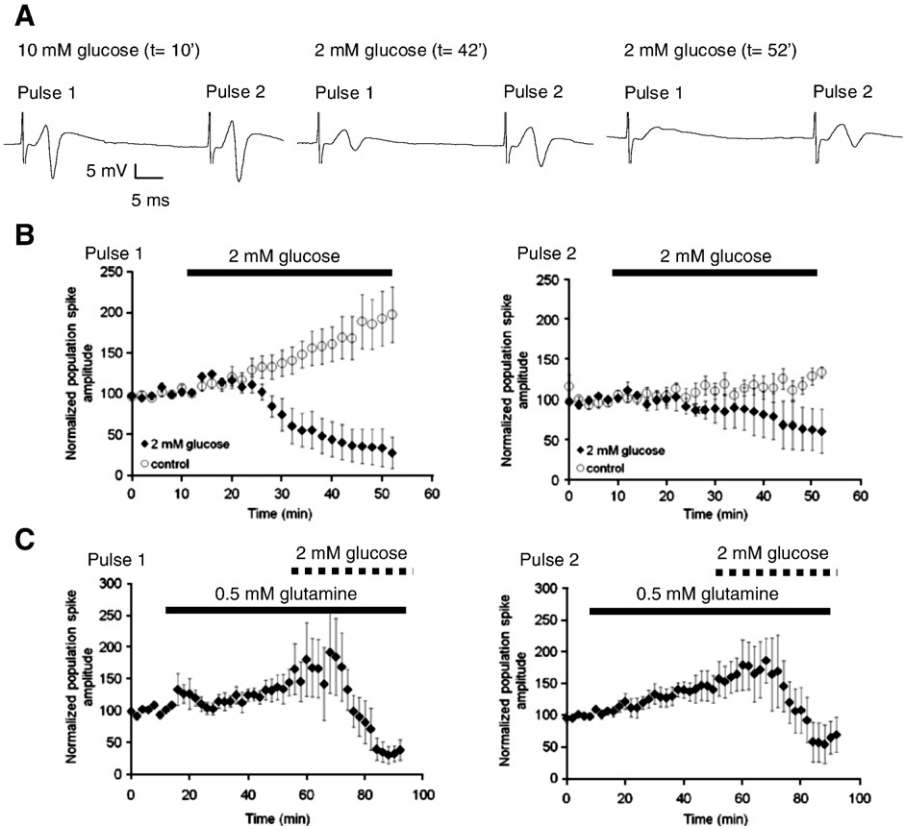

Fig. 5 – Effect of 2 mM glucose in combination with glutamine on paired-pulse orthodromic population spikes evoked in the CA1 region of hippocampal brain slices. A) Sample traces of 2 mM glucose- treated population spikes. B) Change of glucose concentration from 10 to 2 mM. Population spike amplitude of both pulses is significantly reduced at t = 50 min (n = 6). C) Change of glucose concentration from 10 to 2 mM and the presence of 0.5 mM glutamine. Glutamine does not prevent the population spike attenuation (n = 5).

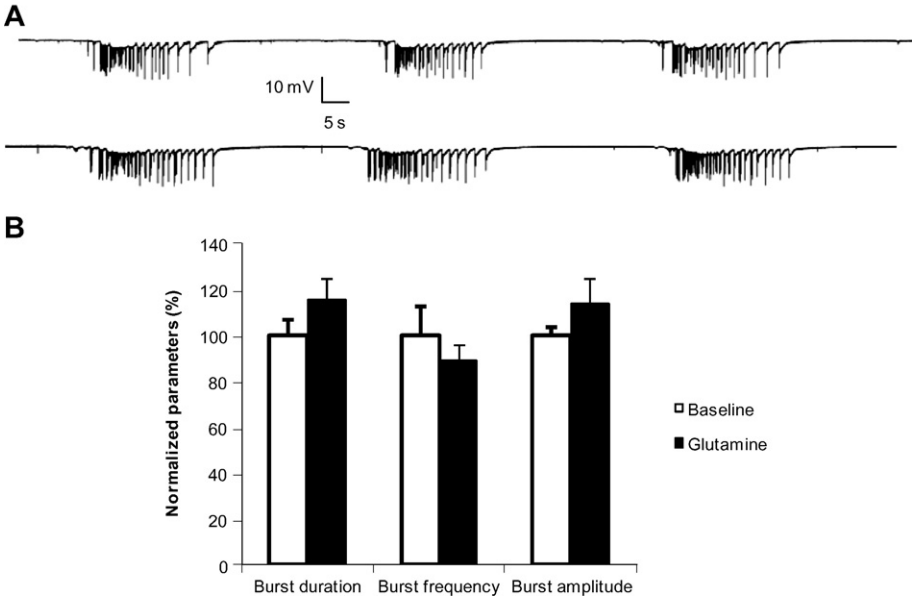

Fig. 6 – Effect of 0.5 mM glutamine on high-K^+ induced epileptiform activity. A) No significant changes were observed between baseline (top trace) and glutamine application (bottom trace, t = 52 min). B) Glutamine does not affect burst duration, amplitude, or frequency compared to baseline (n = 5).

glutamine, if it is present, from the ACSF (Schiff et al., 1985; Szerb and O'Regan, 1985). The high-K^+ activity induced in the present report, however, did not lead to such dependence, at least as manifest in epileptiform waveform over 40 minutes. Glutamine (0.1 to 5 mM) has been shown to protect hippocampal slices against hypoxia while 10 mM glutamine aggravated loss of synaptic function (Schurr et al., 1987). Kam and Nicoll (2007) reported an effect on hippocampal slice synaptic function only at relatively high (4 mM) glutamine levels and after >4 hours; they verified this insensitivity was not limited by slice diffusion kinetics (Fig. 1) by also testing dissociated neurons. ACSF glutamine (<1 mM) increased cortical slice glutamine content and evoked potentials in both normal and hyper-excitable tissue (Tani et al., 2007). Consistent with our findings in hippocampal slices, Tani et al. (2007) did not observe spreading-depression in normal cortical slices after addition of physiological (<1 mM) glutamine to ACSF; however, ≥0.5 mM glutamine promoted spreading-depression in hyper-excitable 'under-cut' cortical slices. In the retinal slice preparation, glutamine (0.5-5 mM) modulates protein synthesis and electrophysiological responses after ~2 hours (Parks et al., 1976; Ames and Nesbett, 1981; Ames and Masland, 1990). Glutamine-containing ACSF (0.6 mM) increased the spontaneous firing rate of most neurons in the ventromedial nucleus of hypothalamic slices (Nishimura et al., 1995). Thus the importance of glutamine in ACSF formulation may depend on the specific experimental paradigm used ('what question is being asked').

3.2. *Role of ACSF glucose*

The attenuation of excitability following glucose reduction is not attributed to osmotic challenge because of the scale of changes and because decreases in glucose/osmolarity would be expected to increase excitability (see Experimental Procedure).

We report that reduction of glucose from 10 mM to 5 mM attenuated pulse 1 after>40 minutes, but there was no significant change in pulse 2 amplitude or paired-pulse ratio even at 50 minutes. Characterizing the dentate region of Guinea-Pig slices, Cox and Bachelard (1982) observed significant decrease in *unpaired* population responses after glucose reduction from 10 to 5 mM. Paired-pulse *ratio*, in the dentate region of rat hippocampal slices, was not compromised by reduction in glucose to from 5 to 1 mM or 10 to 2 mM (Kirchner et al., 2006). We found that reduction in glucose to 2 mM significantly reduced P1 and then P2, but did not significantly change pulse ratios. Fan et al. (1988) and Schurr et al. (1989) reported a decrease in spike amplitude with <2 mM ACSF glucose using single and paired-pulses, but did not examine paired-pulse ratios. Our results are thus consistent with, and reconcile, these previous studies. Across slice neurophysiological studies, 10 mM ACSF glucose maximizes responses to 'physiological' stimuli (and no *attenuation* was reported after *increasing* ACSF glucose from 5 to 10 mM); thus 10 mM ACSF glucose may be a "conservative" baseline concentration to maximize typical slice neurophysiological responses. This result serves as a rational guideline for ASCF formulation, but does not necessarily inform *in vivo* glucose regulation mechanisms (see below).

Less than 2 mM ACSF glucose fails to support specific brain slice function indicators. However, *in vivo* extracellular glucose concentration is in the range of 0.47-4.4 mM (Silver and Erecinska, 1994; McNay and Gold, 1999; Kirchner et al., 2006), thus 2 mM glucose is within the physiological CSF range. One plausible explanation for this considerable discrepancy is that the distance for metabolite diffusion in brain slice (set by slice thickness; >300 μM) is generally higher than the distance *in vivo* (set by intercapillary distance, ~50 μm, LaManna et al., 2004). The 'U' shaped concentration profile of metabolites in a slice (Fujii et al., 1982; Schiff and Somjen 1985) may require that glucose concentration at the *surface* of the slice (set by ACSF formulation) be maintained above physiological concentration to ensure adequate glucose delivery at the slice core. Important from the perspective of ACSF formulation, the relatively high glucose concentration near the slice surface may not adversely affect neuronal function in this region; increasing glucose from 5 to 10 mM does not *decrease* overall slice excitability.

3.3. *ACSF formulation and the brain slice preparation: experimental design*

As illustrated in the cases of hyperglycemic aggravation of neuronal damage (Schurr et al., 1999b; Mehta 2003; Payne et al., 2003) and hypoglycemic associated seizures (Kirchner et al., 2006), the (systemic) effects of compounds *in vivo* may differ categorically from their effects on brain slices. Particularly in the case of metabolites, such differences may be attributed to fundamental differences in neuronal microenvironment regulation (Fig. 1). The results of the present study directly inform brain slice experiment design and interpretation, rather than *in vivo* metabolic dynamics.

The above discussion reinforces the need to consider both fundamental differences between *in vivo* and *in vitro* conditions and specific brain slice experimental paradigms ('what question is being asked') when determining ACSF formulation. Considering the significant differences between *in vivo* and *in vitro* conditions (Fig. 1), disparities in metabolic activity and chemical microenvironment are expected; moreover, the role of glucose and glutamine concentrations in ACSF will presumably vary depending on the experimental conditions including high frequency stimulation, bath temperature, protocol time (>2 hrs), slice thickness, animal species (Cox and Bachelard, 1982; Schiff et al., 1985; Newman et al., 1991; Bacci et al., 2002). Our results support ACSF formulation approaches that methodically deviate from CSF levels to adequately maintain brain slice function. ACSF glucose levels may 'conservatively' be maintained at 10 mM (presumably reflecting limited slice diffusion), while varied benefits of supplementing (0.5 to 4 mM) glutamine may be balanced against increased risk of spreading-depression (presumably reflecting reversing diffusion gradients).

4. Experimental procedure

Transverse hippocampal slices (350 μm) were prepared from male Sprague-Dawley rats (125–150 g); anaesthetized with intraperitoneal ketamine (7.4 mg kg^{-1}) and xylazine (0.7 mg kg^{-1}) and killed by cervical dislocation (Radman et al., 2007). A total of 30 animals have been used and no more than 3 slices from the same animal were used for any experimental condition. The

slices were stored in a holding chamber submerged in "normal" ACSF consisting of (mM): 125 NaCl, 26 $NaHCO_3$, 3 KCl, 1.6 $CaCl_2$, 1.5 $MgSO_4$, 1.25 NaH_2PO4, and 10 glucose, bubbled with a mixture of 95% O_2–5% CO_2. After >60 min, slices were transferred to an interface recording chamber at 34 °C.

Extracellular recordings of population responses in the CA1 pyramidal cell region were made using glass micropipettes (2-8 MΩ) filled with 150 mM NaCl. Bipolar platinum-iridium stimulating electrodes (Merrill et al., 2005) were placed in Schaffer collaterals (orthodromic stimulation) and paired-pulses (30 ms interpulse delay, 0.2 ms pulse duration) were evoked once every 2 minutes. Slices were stimulated with intensity<500 μA (2200 and 2300; A-M Systems, Carlsborg, WA, USA) to produce first population spike amplitude between 1-15 mV and adjusted to 25-75% of the maximum response. Unless otherwise stated, superfusate changes were applied (at t=12 min) for 40 minutes after a baseline was established. Baseline was established as <±10% change in first population spike amplitude over 12 minutes. For each experimental trial, the first (P1) and second (P2) population spike amplitudes were normalized to the respective averages during the baseline period. Paired-pulse faciliatation (PPF) was determined as P2/P1 greater than 1, while Paired-pulse depression (PPD) was determined as P2/P1 less than 1. Paired-pulse ratios (P2/P1) were normalized to the averages during the baseline period of each trial. Results are reported as normalized percentage±standard error; n=number of slices. Student's t-test was performed compared to the normalized control values at t=50 min unless otherwise indicated and the null-hypothesis was rejected if $p<0.05$. We identified outliers using boxplots (Gurcan et al., 1997; Gruetter et al., 2001).

Extracellular recording of epileptiform activity in the CA1 pyramidal cell region were made using glass micropipettes (2-8 MΩ) filled with 150 mM NaCl. After 30 minutes of perfusion with 'high K^{+}' ACSF (125 NaCl, 26 $NaHCO_3$, 8 KCl, 1.0 $CaCl_2$, 1.5 $MgSO_4$, 1.25 NaH_2PO4, and 10 glucose), complete mechanical lesions were made, in the CA2 region, across the Schaffer collateral pathway (Jensen and Yaari, 1988).

The osmolarity of ACSF was measured using a Vapro-5520 vapor pressure osmometer (Wescor, Logan, UT, USA). The osmotic difference between high-K^{+} glutamine absent (308±1 mOsm/L) and high-K^{+} plus 0.5 mM glutamine (310±1 mOsm/L) is significantly less than that previously shown to modulate epileptiform waveform (Andrew et al., 1989; Traynelis and Dingledine, 1989; Rosen and Andrew 1991; Saly and Andrew 1993). The osmotic differences between "normal" ACSF (295±2 mOsm/L), ACSF plus 0.5 mM glutamine (295±3 mOsm/L), and ACSF with 2 mM Glucose (291±2 mOsm/L) are not expected to significantly modulate excitability (Rosen and Andrew 1991; Saly and Andrew 1993; Azouz et al., 1991).

All signals were amplified, and low-pass filtered (1–10 kHz) with an Axoclamp-2B (Axon Instruments, Union City, CA, USA) and FLA-01 amplifiers (Cygnus Technology, Delaware Water Gap, PA, USA); then digitized and processed using a Power 1401 and Signal software (CED Cambridge Electronic Design, Cambridge, UK).

It is important to note that neither population spike amplitude nor epileptiform waveform is not necessarily indicative of a "healthier" slice; however, these extracellular field waveforms are established and quantitative indicators of gross brain slice function. Changes in neuronal excitability, metabolism, or excitatory or inhibitory synaptic function (including pre- or post-synaptic effects) would be manifest in these signals (Schwechter et al., 2003); specifically using a 30 ms interpulse delay and the high-K^{+} model (Korn et al., 1987; Traynelis and Dingledine, 1988; Patrylo et al., 1994; Leschinger et al., 1993). These metrics are therefore used in the context of this study to access any effects of ACSF organics on gross slice function. In addition, monitoring of evoked (paired) population spikes and epileptiform activity is ubiquitous across slice studies on normal and pathological brain function, such that the results of this paper using these metrics directly impact the appropriate design of such studies.

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