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BRAIN RESEARCH

Research Report

Contribution of calpain activation to early stages of hippocampal damage during oxygen–glucose deprivation

Michael Grammer, Dong Li, Nirubhana Arunthavasothy, Janusz Lipski*

Department of Physiology, Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Grafton, Auckland 1023, New Zealand

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ABSTRACT

Calpains are Ca^{2+}-activated enzymes which cleave cytoskeletal and other proteins, contributing to neuronal damage in conditions of pathological intracellular Ca^{2+} elevation, including stroke. However, the consequences of calpain overactivation have typically been observed hours after insult. To identify the earliest events attributable to calpain activation, and thus potentially isolate calpain substrates involved in acute neuronal damage, we dynamically recorded the effects of calpain inhibition in an in vitro model of stroke. Extracellular DC potentials and fEPSPs were monitored together with changes of light transmittance (as a measure of cell and mitochondrial swelling) and Rh 123 fluorescence (to monitor mitochondrial membrane potential; $\Delta\Psi_m$) in hippocampal slices obtained from P12–P17 rats. No differences were observed in the latencies of fEPSP disruption or onset of extracellular DC shifts associated with hypoxic spreading depression (HSD) evoked by oxygen–glucose deprivation (OGD) under control conditions or in the presence of calpain inhibitor III (MDL 28170). However, a significant difference was observed in transmitted light signals during OGD with calpain inhibition. Given the potential contribution of mitochondrial swelling to changes in light transmittance, these experiments were also conducted in the presence of cyclosporin A to block opening of the mitochondrial permeability transition pore (MPTP). Our results indicate that differences in OGD-induced changes of light transmittance in the presence of MDL 28170 are not likely the result of MPTP blockade or changes in dendritic beading. We propose that calpain inhibition may alter changes in light transmittance by limiting conformational changes of mitochondria.

1. Introduction

During ischemia/reperfusion, neurons undergo a series of physiological events leading to cell death or damage. This neurological injury is frequently modelled in vitro by oxygen–glucose deprivation (OGD) (Lipski et al., 2006; Somjen, 2001). In the absence of oxygen and glucose, ATP levels are quickly depleted and cellular ionic gradients are lost. The equilibration of ions across the plasma membrane leads to neuronal damage through elevated intracellular calcium concentration as well as excessive cell and mitochondrial swelling. Paradoxically, restoring oxygen and glucose to hypoxic tissue (a condition mimicking reperfusion) initiates additional neurotoxic responses. This 'reperfusion injury' is largely attributed

* *Corresponding author*. Fax: +64 9 373 7499.
E-mail address: m.grammer@auckland.ac.nz (M. Grammer).

Abbreviations: CsA, cyclosporin A; HSD, hypoxic spreading depression; MPTP, mitochondria permeability transition pore; OGD, oxygen–glucose deprivation; Rh 123, Rhodamine 123; ROI, region of interest; ROS, reactive oxygen species; $\Delta\Psi_m$, mitochondrial membrane potential

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to a burst of reactive oxygen species (ROS) generated by mitochondria and/or cytosolic oxidases that damages membrane lipids as well as cellular proteins and nucleic acids (Abramov et al., 2007; Nicholls and Budd, 2000). ROS production during reperfusion is further stimulated by elevated intracellular calcium levels (Nicholls and Budd, 2000).

Excessive intracellular calcium during ischemia/reperfusion or OGD damages neurons through multiple pathways, among which activation of calpains appears to play a major role. Calpains comprise a family of 14 calcium-activated cysteine proteases that, upon activation, trigger substrate-specific proteolysis resulting in degradation, activation or translocation of the substrate (Carragher, 2006; Goll et al., 2003). Following brief ischemic episodes, calpain activation (detected as the breakdown product of a preferred substrate, spectrin) was reported in CA1 pyramidal neurons (Seubert et al., 1989). Following longer ischemic events, calpain activation was observed in other, progressively vulnerable brain regions (Roberts-Lewis et al., 1994). Neuroprotection from ischemia/reperfusion by calcium-limiting agents, such as the NMDA channel blocker MK-801, correlates with decreased calpain activation (Roberts-Lewis and Siman, 1993; Seubert et al., 1990; Zhou and Baudry, 2006). In addition, calpain inhibitors provide neuroprotection from ischemia both in vitro (Malagelada et al., 2005; Newcomb-Fernandez et al., 2001) and in vivo (Hong et al., 1994; Kawamura et al., 2005; Lee et al., 1991).

While inhibition of calpains clearly provides neuroprotection from ischemia/reperfusion, as well as other pathological conditions associated with calcium dysregulation, the specific substrates of calpain-mediated proteolysis responsible for neuronal damage under these conditions have not yet been identified. There are currently over 100 calpain substrates including ion channels/pumps, membrane anchoring proteins, enzymes and a large number of cytoskeletal/membrane associated proteins. Given the involvement of cell and mitochondrial swelling during the early stages of ischemia (Christophe and Nicolas, 2006; Kimelberg, 1995), and recent evidence that calpains may target mitochondria during ischemia (Chen et al., 2002), we hypothesized that calpain inhibition might decrease or slow the onset of cellular or mitochondrial swelling, thus implicating cytoskeletal and/or mitochondrial associated calpain substrates in early ischemic damage. The identification of synaptic and membrane proteins that support synaptic transmission and cell membrane potential as calpain substrates (Khoutorsky and Spira, 2005; Vanderklish et al., 1995) also suggests that calpain activation might influence electrophysiological responses during ischemia and reperfusion.

To resolve the potential contribution of calpain activation during early stages of cellular damage caused by ischemia/reperfusion, electrophysiological as well as intrinsic and fluorescent optical signals in the CA1 region of the hippocampus were dynamically recorded during OGD and reperfusion. Cellular and mitochondrial swelling associated with OGD/reperfusion alters the optical properties of neural tissue which were recorded using conventional light microscopy. In addition, we simultaneously monitored changes in mitochondrial membrane potential ($\Delta\Psi_m$) with the fluorescent indicator Rhodamine 123 (Rh 123).

2. Results

2.1. *Effects of calpain inhibition during OGD and reperfusion*

One of the earliest events in ischemia, or during OGD regarded as a model of ischemic damage, is the disruption of synaptic transmission (Lipski et al., 2006; Schiff and Somjen, 1987). In agreement with this finding, we observed rapid decay of synaptic transmission between Schaeffer collaterals and pyramidal CA1 neurons, measured as the loss of fEPSPs in stratum (Str) radiatum within 2.5 min of OGD (Fig. 1A). Calpain inhibitor III (MDL 28170; 40 μM) did not alter the latency to synaptic disruption (Fig. 1C; $p=0.46$), which corresponds with previous reports of adenosine-mediated inhibition as the principle mechanism of synapse blockade during OGD (Abbracchio and Cattabeni, 1999).

Another event during ischemia or OGD is hypoxic spreading depression (HSD) associated with a large redistribution of

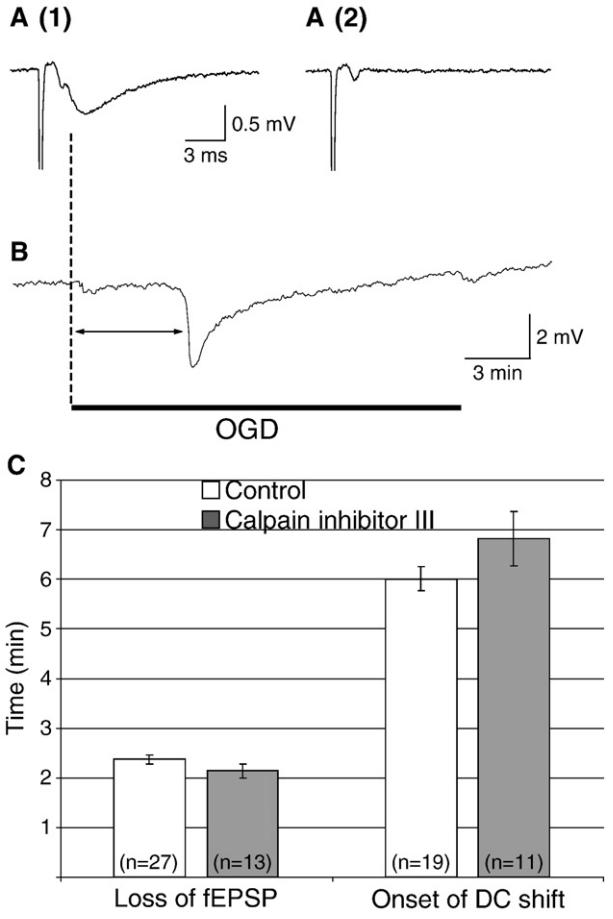

Fig. 1 – Electrophysiological responses to OGD and calpain inhibition. A: Examples of fEPSP recordings before (A1) and after (A2) 3 min of OGD. Each trace represents the average of 6 sweeps. B: Extracellularly recorded DC shift observed 6 min after the onset of OGD (double-headed arrow). C: Times (mean ± S.E.M.) at which the loss of fEPSPs and extracellular DC shifts occurred during OGD under control conditions or in the presence of MDL 28170 (40 μM).