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Research Report

Divergent effects of painful nerve injury on mitochondrial Ca^{2+} buffering in axotomized and adjacent sensory neurons

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ABSTRACT

Mitochondria critically regulate cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$), but the effects of sensory neuron injury have not been examined. Using FCCP (1 μM) to eliminate mitochondrial Ca^{2+} uptake combined with oligomycin (10 μM) to prevent ATP depletion, we first identified features of depolarization-induced neuronal $[Ca^{2+}]_c$ transients that are sensitive to blockade of mitochondrial Ca^{2+} buffering in order to assess mitochondrial contributions to $[Ca^{2+}]_c$ regulation. This established the loss of a shoulder during the recovery of the depolarization (K^+)-induced transient, increased transient peak and area, and elevated shoulder level as evidence of diminished mitochondrial Ca^{2+} buffering. We then examined transients in Control neurons and neurons from the 4th lumbar (L4) and 5th lumbar (L5) dorsal root ganglia after L5 spinal nerve ligation (SNL). The SNL L4 neurons showed decreased transient peak and area compared to control neurons, while the SNL L5 neurons showed increased shoulder level. Additionally, SNL L4 neurons developed shoulders following transients with lower peaks than Control neurons. Application of FCCP plus oligomycin elevated resting $[Ca^{2+}]_c$ in SNL L4 neurons more than in Control neurons. Whereas application of FCCP plus oligomycin 2 s after neuronal depolarization initiated mitochondrial Ca^{2+} release in most Control and SNL L4 neurons, this usually failed to release mitochondrial Ca^{2+} from SNL L5 neurons. For comparable cytoplasmic Ca^{2+} loads, the releasable mitochondrial Ca^{2+} in SNL L5 neurons was less than Control while it was increased in SNL L4 neurons. These findings show diminished mitochondrial Ca^{2+} buffering in axotomized SNL L5 neurons but enhanced Ca^{2+} buffering by neurons in adjacent SNL L4 neurons.

Abbreviations: $\Delta\Psi_m$, inner mitochondrial membrane potential; $[Ca^{2+}]_c$, cytoplasmic Ca^{2+} concentration; DRG, dorsal root ganglion; ER, endoplasmic reticulum; FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; L4, 4th lumbar; L5, 5th lumbar; PMCA, plasma membrane Ca^{2+}-ATPase; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; SOCE, store-operated Ca^{2+} entry; SNL, spinal nerve ligation; TMRM, tetramethylrhodamine methyl ester

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1. Introduction

Pain following peripheral nerve injury is in part the result of elevated sensory neuron excitability due to disordered Ca^{2+} signaling. Specifically, axonal trauma is followed by diminished Ca^{2+} influx through voltage-gated Ca^{2+} channels (Hogan et al., 2000; McCallum et al., 2006), in part attributable to reduced activation of Ca^{2+}-calmodulin-dependent protein kinase II (Kawano et al., 2009; Kojundzic et al., 2010; Tang et al., 2012). The cytoplasmic Ca^{2+} signal initiated by Ca^{2+} influx is further shaped in sensory neurons by the simultaneous processes of Ca^{2+} extrusion, sequestration, and release from stores. We have identified dysfunction of these processes in axotomized neurons following painful nerve injury, including elevated function of the plasma membrane Ca^{2+}-ATPase (PMCA) (Gemes et al., 2012), accompanied by decreased resting cytoplasmic Ca^{2+} ($[Ca^{2+}]_c$) (Fuchs et al., 2005), reduced function of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Duncan et al., 2013) that reduces endoplasmic reticulum (ER) Ca^{2+} stores (Gemes et al., 2009; Rigaud et al., 2009), with resulting elevation of store-operated Ca^{2+} entry (SOCE) (Gemes et al., 2011) and diminished release of Ca^{2+} from stores upon neuronal activity through the process of Ca^{2+}-induced Ca^{2+} release. Together, these disturbances of Ca^{2+} signaling contribute to elevated generation and transmission of high-frequency trains of action potentials in the injured sensory neurons (Gemes et al., 2009, 2013; Hogan et al., 2008; Lirk et al., 2008; Sapunar et al., 2005; Tang et al., 2012).

Mitochondria are also recognized as a critical element in intracellular Ca^{2+} management (Nicholls, 2005). In sensory neurons, mitochondria serve as a nonsaturable buffer with a clearance rate in neurons that exceeds the PMCA and Na^+/Ca^{2+} exchanger (Herrington et al., 1996). Mitochondrial sequestering of Ca^{2+} during periods of high $[Ca^{2+}]_c$ after neuronal activation buffers peak $[Ca^{2+}]_c$, while the subsequent slow release of Ca^{2+} as the $[Ca^{2+}]_c$ falls prolongs $[Ca^{2+}]_c$ recovery and produces a characteristic shoulder in the Ca^{2+} transient (Werth and Thayer, 1994). Thus, mitochondrial Ca^{2+} buffering modulates the duration and peak levels of activity-induced Ca^{2+} transients, and protects the neuron from excessive elevations of $[Ca^{2+}]_c$. More recent findings indicate constitutive operation of mitochondrial Ca^{2+} cycling even at low levels of $[Ca^{2+}]_c$ in resting sensory neurons (Colegrove et al., 2000; Kang et al., 2008). The ability of mitochondria to sequester Ca^{2+} is tied to their energy state, since Ca^{2+} influx through the mitochondrial Ca^{2+} uniporter is driven by the potential difference across the inner mitochondrial membrane ($\Delta\Psi_m$). In a reciprocal fashion, mitochondrial energy production in sensory neurons is upregulated by Ca^{2+} loading (Duchen, 1999), which provides a link between cell activity and energy production through elevations of $[Ca^{2+}]_c$.

There has been minimal exploration of mitochondrial function in sensory neurons subjected to models of painful traumatic neuropathy. We have previously noted a decrease in the incidence of a shoulder (also called a plateau) in the depolarization-induced transient trace of axotomized fifth lumbar (L5) neurons in the spinal nerve ligation (SNL) model of peripheral nerve injury (Fuchs et al., 2005), which suggests a deficit in mitochondrial function after injury. Furthermore, there is a growing recognition of a contributing role of mitochondrial dysfunction in painful neuropathy (Chu et al., 2011; Flatters and Bennett, 2006; Joseph and Levine, 2006). Accordingly, we hypothesized that nerve injury disrupts Ca^{2+} buffering by mitochondria in axotomized sensory neurons. Ideally, mitochondrial buffering of activity-induced Ca^{2+} influx would be gauged by isolating this process through blocking SERCA and PMCA, but this leads to inability of the neuron to maintain stable $[Ca^{2+}]_c$ under resting conditions (Duncan et al., 2013). We therefore adopted the strategy of first identifying measurable features of mitochondrial buffering revealed by their sensitivity to $\Delta\Psi_m$ elimination with FCCP. These features were then compared in injured and control neurons, including the L4 population of neurons that are intact after SNL but are exposed to inflammation that accompanies Wallerian degeneration of the degenerating L5 axonal fragments. Overall, our findings confirm a fundamental dependence of sensory neuron Ca^{2+} homeostasis on mitochondrial activity. Furthermore, we have identified extensive disruption of this role in divergent patterns for both axotomized (L5) and intact (L4) neuronal populations after SNL, such that the axotomized L5 neurons exhibit features suggesting reduced mitochondrial Ca^{2+} buffering while the L4 population shows amplified mitochondrial buffering activity.

2. Results

2.1. FCCP plus oligomycin for blockade of mitochondrial Ca^{2+} buffering

As an overall strategy for evaluating injury effects on mitochondrial Ca^{2+} buffering, we first sought to characterize which quantifiable features of the depolarization-induced Ca^{2+} transient (Fig. 1A and B) reliably reflect mitochondrial Ca^{2+} uptake and release. This requires selective blockade of mitochondrial Ca^{2+} buffering, for which we chose the protonophore FCCP (1 μM) since this prevents Ca^{2+} accumulation by elimination of $\Delta\Psi_m$. However, protonophores also deplete ATP through reversal of ATP synthase function (Budd and Nicholls, 1996), which may disrupt ATP-dependent SERCA and PMCA pathways, resulting in nonspecific inhibition of Ca^{2+} sequestration and extrusion. We therefore co-administered the ATP synthase blocker oligomycin (10 μM), which has been shown to prevent ATP depletion in dissociated neurons (Budd and Nicholls, 1996). To further confirm preservation of ATP levels in our dissociated sensory neurons, we assayed the level of ATP available for driving physiological functions by measuring the performance of the ATP-driven Ca^{2+} pump PMCA as an indicator of ATP availability (Schatzmann and Vincenzi, 1969), quantified as the time constant of recovery for transients during SERCA blockade (thapsigargin 1 μM, Fig. 1C) (Gemes et al., 2012). This was unaffected by this combination of FCCP and oligomycin (FCCP/Oligo, Fig. 1D), which confirms prior findings that FCCP/Oligo does not deplete ATP during the timeframe of our experiments (Budd and Nicholls, 1996). Using TMRM as an indicator of $\Delta\Psi_m$, we additionally confirmed the efficacy of