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Selective dendritic susceptibility to bioenergetic, excitotoxic and redox perturbations in cortical neurons ☆

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ABSTRACT

Neurodegenerative and neurological disorders are often characterised by pathological changes to dendrites, in advance of neuronal death. Oxidative stress, energy deficits and excitotoxicity are implicated in many such disorders, suggesting a potential vulnerability of dendrites to these situations. Here we have studied dendritic vs. somatic responses of primary cortical neurons to these types of challenges in real-time.

Using a genetically encoded indicator of intracellular redox potential (Grx1-roGFP2) we found that, compared to the soma, dendritic regions exhibited more dramatic fluctuations in redox potential in response to sub-lethal ROS exposure, and existed in a basally more oxidised state. We also studied the responses of dendritic and somatic regions to excitotoxic NMDA receptor activity. Both dendritic and somatic regions experienced similar increases in cytoplasmic Ca^{2+}. Interestingly, while mitochondrial Ca^{2+} uptake and initial mitochondrial depolarisation were similar in both regions, secondary delayed mitochondrial depolarisation was far weaker in dendrites, potentially as a result of less NADH depletion. Despite this, ATP levels were found to fall faster in dendritic regions. Finally we studied the responses of dendritic and somatic regions to energetically demanding action potential burst activity. Burst activity triggered PDH dephosphorylation, increases in oxygen consumption and cellular NADH:NAD ratio. Compared to somatic regions, dendritic regions exhibited a smaller degree of mitochondrial Ca^{2+} uptake, lower fold-induction of NADH and larger reduction in ATP levels. Collectively, these data reveal that dendritic regions of primary neurons are vulnerable to greater energetic and redox fluctuations than the cell body, which may contribute to disease-associated dendritic damage. This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

1. Introduction

In many chronic and acute conditions of the brain involving neuronal dysfunction there is substantial evidence that the effects of a diverse array of disease-causing events, agents and mutations at least partly converge on a common set of consequences centred on excitotoxicity, energy imbalance, oxidative stress and mitochondrial dysfunction [55,70,71,74,91].

Excitotoxicity is caused by the global or local accumulation of glutamate, leading to inappropriate activity of the NMDA subtype of glutamate receptor (NMDAR) which mediates Ca^{2+}-dependent cell death and dysfunction [17,61]. This 'excitotoxicity' is a major contributor to neuronal loss and dysfunction in acute neurological disorders including stroke and traumatic brain injury [60]. However, more local, progressive excitotoxicity is also implicated in the pathogenesis of neurodegenerative disease [62,80]. For example, synaptic loss in Alzheimer's disease (AD) models induced by oligomeric Aβ is thought to proceed at least in part via a local NMDAR-dependent excitotoxic mechanism [53,66]. Huntington's disease is also a disorder associated with aberrant NMDAR activity and excitotoxicity [29], in part due to an elevation of extrasynaptic NMDAR activity which promotes neuronal dysfunction as well as preventing non-toxic mHtt inclusion formation [72,77].

The principle cause of excitotoxicity in stroke is a loss of bioenergetic homeostasis, leading to dysfunctional glutamate uptake mechanisms and accumulation of extracellular glutamate. Furthermore there is considerable evidence that energetic and metabolic perturbations occur in chronic neurodegenerative diseases, not just acute disorders such as stroke or traumatic brain injury [58]. Central to energy homeostasis, of course, is the requirement that functional mitochondria are able to respond to the changing demands of a neuron (for example during periods of strong synaptic activity). Dysfunctional mitochondria are a hall-mark of many chronic and acute neurological disorders, which can be triggered by both excitotoxic and non-excitotoxic mechanisms. An example of the latter is oxidative stress, which is not only triggered by excitotoxicity and a variety of disease-causing agents and mutations, but can also be further exacerbated by mitochondrial dysfunction.

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Given that neurons are highly polarised cells with many spatially and biochemically distinct regions, it is perhaps not surprising that these regions are differentially affected under pathological conditions. For example, there is good evidence that changes in dendritic and axonal morphology and properties take place in neurodegenerative disease in advance of neuronal loss [65]. In AD and mouse models of AD, dystrophic neurites and spine alterations correlate with proximity to amyloid plaques [35,87]. Moreover, in animal models of AD dendritic alterations have been observed in advance of plaque formation [84] and recent evidence suggests that structural dendritic deficits in an AD model are functionally linked to hyperexcitability [85]. α-Synuclein inclusions promote mitochondrial oxidative stress in dopaminergic neurons that is higher in dendritic regions than in the soma, suggestive of dendritic vulnerability in Parkinson's disease [25]. Even normal ageing is associated with a reduction in dendritic complexity and spine numbers [23]. Acute excitotoxicity such as exposure to NMDA or oxygen–glucose deprivation also triggers early dendritic alterations in the form of neuritic beads [36], although their role (protective or pathological) is not well understood.

Despite this, we have an incomplete picture of how dendritic vs. somatic regions respond in real-time to metabolic, oxidative and excitotoxic stress. This knowledge could help understand the types of insult that trigger selective vulnerability to more peripheral regions of a neuron, as well as indicate whether classical neuroprotective strategies are appropriate for preventing more localised dendritic or synaptic damage. Key to gaining a better understanding of real-time responses of neurons to various forms of stress has been the development of an expanding array of genetically encoded indicators designed to report on a variety of metabolic intermediates, second messenger levels, and signal pathway activity. Particularly important for studying dynamic neuronal responses to excitotoxic, metabolic and oxidative stress include mitochondrially targeted Ca^{2+} indicators, probes for NADH:NAD ratio, ATP levels, AMPK activity and cellular redox state (GSH:GSSG ratio). These indicators can be usefully employed in parallel with more classical imaging techniques, such as charged dye reporting of mitochondrial membrane potential, and NAD(P)H autofluorescence measurements.

Here we have employed these existing probes, plus a newly targeted version of GCaMP2, to compare dendritic vs. somatic responses of cortical neurons to excitotoxic insults (lethal and sub-lethal), a sub-lethal oxidative challenge, and finally the metabolic demands of intense synaptic activity. As well as illustrating the utility of these probes, we found that compared to the soma, dendrites were more susceptible to strong fluctuations in redox state and ATP levels. However, dendritic mitochondria appeared more resistant to catastrophic depolarisation during exposure to excitotoxic insults.

2. Results

2.1. Analysis of subcellular redox potential changes following H_2O_2 exposure

We first decided to compare dendritic vs. somatic responses to an oxidative challenge. Classical probes are based on non-fluorescent dyes which, upon oxidation, become fluorescent. However, this is a one-way reaction and so does not indicate recovery of redox status. To visualise the redox potential of the cell directly, we transfected neurons with a vector encoding the Grx1-roGFP2 genetically encoded reporter of the glutathione redox potential [42] (Fig. 1A). The 390/480 nm excitation ratio of Grx1-roGFP2 is highly sensitive to small fluctuations in redox potential. Its dynamic range (−320 to −240 mV [42]) means that small changes in the degree of GSH oxidation lead to large changes in Grx1-roGFP2 signal [42] and so it is ideal for measuring cellular responses to sub-toxic oxidative challenges. We found that low doses of H_2O_2 produced strong fluctuations in the probe signal which were frequently saturated at above 25 μM H_2O_2. Using a H_2O_2 dose well within the dynamic range (10 μM H_2O_2) we monitored the fluctuation in glutathione redox potential in somatic vs. dendritic regions, normalised to the maximal response elicited by a high dose of H_2O_2 (100 μM). We found that dendritic regions exhibited stronger perturbations to the glutathione redox potential, compared to the soma of that same cell. Fig. 1B shows an example recording from the dendritic and somatic regions of a single cell, and Fig. 1C shows the full data set, and Fig. 1E shows the 10 μM H_2O_2-induced change in both regions. We also observed that dendritic Grx1-roGFP2 ratios tended to be higher in dendritic regions compared to their soma, suggesting an elevated basal oxidation state. To test this, in a set of cells we treated them with DTT to provide highly reducing conditions in order to minimize the 390/480 nm excitation ratio. We found that the effect of DTT was greater in dendritic regions than in somatic regions, suggesting that dendrites are indeed in an elevated basal oxidation state.

2.2. Analysis of subcellular metabolic and mitochondrial changes during excitotoxicity

We next investigated somatic and dendritic responses to excitotoxic Ca^{2+} influx through the NMDA receptor (NMDAR). We first studied the consequences of excitotoxic NMDAR activity for cellular ATP levels in dendritic and somatic regions. We used the genetically encoded ATP FRET probe AT1.03 [52], which shows a YFP/CFP emission ratio of around 1.6 in resting neurons, and which falls to around 0.6 upon depletion of cellular ATP achieved by treatment with the mitochondrial uncoupler FCCP (data not shown). Application of NMDA (100 μM) elicited similar increases in cytoplasmic Ca^{2+}, measured using the cytoplasmic indicator GCaMP2 (Fig. 2A). However, we found that the rate of loss of ATP, as measured using AT1.03, was more rapid in dendritic regions than in the soma over early time points (Fig. 2B, C).

Loss of mitochondrial membrane potential (Ψm) is an early event in excitotoxicity, initially triggered by mitochondrial Ca^{2+} uptake [1,82], but later irreversible and Ca^{2+}-independent, involving PARP-mediated depletion of NADH [1]. We therefore decided to investigate whether the mitochondria in these different regions respond differently to excitotoxic Ca^{2+} influx. Using the Ψm probe rhodamine-123 (Rh123), we studied the loss of Ψm in response to bath application of NMDA. Rh123 partitions into the polarised mitochondria of neurons where its accumulation causes self-quenching. When the mitochondria depolarise during exposure to excitotoxic insults, Rh123 moves increasingly into the cytoplasm, whereupon it de-quenches and its fluorescent signal increases [57,86]. The fluorescence signal is normalised cell-to-cell and region-to-region by calculating the maximal fluorescence at the end of the experiment upon complete mitochondrial depolarisation (achieved by FCCP treatment). We observed an initial slow modest depolarisation in the soma of neurons, followed by a secondary, more dramatic loss of Ψm (Fig. 2D, E). This has been described by others and may involve cellular NADH loss and permeability transition [1]. In dendritic regions however, while the initial slow modest depolarisation was also observed, the secondary loss of Ψm was far less dramatic, and in some cases missing entirely (Fig. 2D, E).

To investigate a potential reason for this, we measured Ca^{2+} uptake into the mitochondria using matrix-targeted mito-GCaMP2, and found no significant differences between uptake in dendritic vs. somatic regions (Fig. 2F). The fact that matrix Ca^{2+} rises to similar levels in the somatic and dendritic mitochondria is consistent with the similar levels of initial mitochondrial depolarisation, but begs the question as to why secondary depolarisation is much stronger in somatic regions. Since NADH depletion is an important mediator of the secondary component of mitochondrial depolarisation [1], differences in NADH depletion offer another explanation. We studied NAD(P)H autofluorescence which, since NADH is in excess over NADPH, primarily reports NADH levels. As with previous studies, we observed that NADH autofluorescence was concentrated in the mitochondria throughout the neuron. We found that the proportional drop in NADH in somatic regions was

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