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P01.53. Spheroid formation and axonal severing in adult neurons during oxidative stress: role of calcium

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Purpose

Axonal severing is critical to the irreversible disability that occurs over the course of multiple sclerosis (MS). Reactive oxygen species (ROS) are implicated in neurodegenerative aspects of MS: axonal spheroid formation, severing, and axoplasmic Ca^{2+} elevation. However, the exact role of Ca^{2+} in spheroid formation remains unclear. The mechanism of action of natural anti-oxidants such as lipoic acid, which provide neuroprotection during oxidative stress in MS model, also remains unclear.

Methods

Primary cortical neurons from adult mice were subjected to physiologically-relevant levels of H_2O_2. Ca^{2+} dynamics and its sources were examined during spheroids formation using real time imaging, ratiometric Ca^{2+} indicators and immunocytochemistry.

Results

Exposure to ROS led to a 3.5 fold increase in axoplasmic Ca^{2+} by 30 min. Onset of axonal spheroid formation began at 15 min when Ca^{2+} increase was 2.2 fold. Axonal severing occurred at sites of spheroids around 90-120 min. Analysis of small axonal segments revealed an uneven distribution of Ca^{2+} during exposure to H_2O_2. Micrometers apart, focal Ca^{2+} increases in small axonal domains ranged from 2.8 to 4.4 fold. Domains with a 3.8 to 4.4-fold increase correlated with the sites of spheroids, suggesting high focal extracellular Ca^{2+} influx at these sites. Several treatments significantly attenuated Ca^{2+} increase and completely abolished spheroid formation under ROS: removal of extracellular Ca^{2+}; N-type Ca^{2+} channel blocker omega-conotoxin GVIA; L-type Ca^{2+} channel blocker amlodipine; and reverse Na+/Ca^{2+} exchanger (NCX1) blocker KB-R7943. Aggregation of reverse NCX1 and N-type voltage-gated Ca^{2+} channel was detected at spheroids.

Conclusion

Our results reveal a correlation between focal axoplasmic Ca^{2+} and spheroid formation and suggest that focal aggregation of the reverse NCX1 and N-type Ca^{2+} channel plays central role in high focal Ca^{2+} increase during oxidative stress. These findings provide a basis for investigating the neuroprotective mechanism of the natural anti-oxidant lipoic acid during oxidative stress.

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