Published in final edited form as:

Ann N Y Acad Sci. 2007 March ; 1099: . doi:10.1196/annals.1387.005.

Rapid downregulation of NCX and PMCA in hippocampal neurons following H₂O₂ oxidative stress

Sertac N. Kip and Emanuel E. Strehler

Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN 55905

Abstract

Na⁺/Ca²⁺ exchangers (NCXs) and plasma membrane Ca²⁺ pumps (PMCAs) are crucial for intracellular Ca²⁺ homeostasis and Ca²⁺ signaling. Elevated [Ca²⁺]_i is a hallmark of neurodegenerative disease and stroke. Here we studied the short-term effect of oxidative stress on the plasma membrane Ca²⁺ extrusion systems in hippocampal neurons and found that after 2–3 hours exposure to 300 μM H₂O₂, all NCXs and PMCAs were significantly downregulated at the RNA (NCX) and protein (PMCA) level. Rapid internalization and aggregation of the PMCA was also observed. Our data show that the plasma membrane calcium extrusion systems are sensitive early targets of neurotoxic oxidative stress.

Keywords

Calcium extrusion; hippocampal neurons; Na⁺/Ca²⁺ exchanger; NCX; oxidative stress; PMCA

INTRODUCTION

Oxidative stress plays a crucial role in conditions such as aging, cardiac dysfunction, ischemic injuries, and neurodegenerative diseases including Alzheimer Disease.^{1,2} Neuronal death follows Ca²⁺ dyshomeostasis and the generation of oxidative stress.^{3,4} However, despite studies linking neurotoxicity to Ca²⁺ overload, the relevance of altered Ca²⁺ efflux in the context of neurodegeneration is poorly understood. Because the maintenance and restoration of normal [Ca²⁺]_i is primarily dependent on the proper function of plasma membrane Ca²⁺ extrusion systems comprised of Ca²⁺ pumps (PMCA) and Na⁺/Ca²⁺ exchangers (NCX/NCKX), these Ca²⁺ transporters are primary candidates as early targets of oxidative stress. We therefore determined the effect of exposure to H₂O₂ on the expression of NCX and PMCA in primary rat hippocampal neurons.

MATERIALS AND METHODS

Primary embryonic rat (E18) hippocampal neuron and glial cultures were prepared as described.⁵ The methods for RT-PCR, including the specific primers for amplification of NCX1, NCX2 and NCX3 transcripts, have also been reported.⁵ Western blotting and immunofluorescence confocal microscopy were performed as described, using primary pan-PMCA antibody 5F10 at 1:2,000 and 1:600, respectively.^{5,6} To determine the effects of oxidative stress, cells were treated with 150–600 μM H₂O₂ added to the media for 1–3 hours prior to analysis by RT-PCR, Western, or immunofluorescence microscopy.

RESULTS AND DISCUSSION

We analyzed the effect of the addition of H_2O_2 to the culture media of mature rat hippocampal neurons on the expression of NCX1-3 at the transcript level. Using RT-PCR, we found that a 3-hour treatment with 300 μM H_2O_2 resulted in an almost complete depletion of the transcripts for NCX1 and NCX3, whilst NCX2 transcripts were much less affected (Figure 1). NCX2 is highly expressed in the adult hippocampus, with transcript levels exceeding those of the other NCX isoforms by up to an order of magnitude.⁷⁻⁹ The apparent protection of NCX2 mRNA from H_2O_2 -induced oxidative damage may thus simply reflect the elevated steady-state levels of NCX2 transcripts. However, the data also suggest that NCX2 mRNA levels (and thus perhaps protein function) may be at least partially resistant to moderate oxidative stress. Figure 1 also indicates that NCX transcripts in glia were much less affected by 300 μM H_2O_2 than in primary neurons. Differences in susceptibility to oxidative stress among the different cell types likely account for this effect.

We used Western blotting to determine the effect of H_2O_2 on the expression of the PMCA in hippocampal neurons. Figure 2A shows that there was a dose-dependent reduction in the relative PMCA level. After a 2-hour treatment with 300 μM H_2O_2 , PMCA levels decreased to about 50% of control. The oxidative stress also leads to a selective redistribution and gradual disappearance of PMCA immunoreactivity from the plasma membrane. PMCA was localized mainly in the soma membrane and in punctuate structures in dendrites in mature hippocampal neurons (Figure 2B, left panel). After a 2-hour treatment with 300 μM H_2O_2 , PMCA appeared aggregated in large clusters in what may be degenerating dendrites (Fig. 2B, middle panel). At high levels (600 μM) of H_2O_2 , cells showed reduced PMCA immunoreactivity already after one hour, and the staining concentrated in large clusters.

CONCLUSION

H_2O_2 -mediated oxidative stress leads to a rapid (within a few hours) decrease in the major plasma membrane Ca^{2+} extrusion systems. Functionally, this will result in reduced Ca^{2+} efflux capacity, and thus in a reduced capacity to counteract potentially harmful excitotoxic Ca^{2+} loads. Strategies to protect against loss of NCX and PMCA function may be useful to prevent the progression of the harmful biochemical cascades leading to neurodegeneration following oxidative stress and Ca^{2+} overload.

Acknowledgments

This work was supported by NIH grant RO1 GM28835 and the Mayo Foundation for Medical Education and Research.

References

1. Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. *J Hypertens.* 2000; 18:655–673. [PubMed: 10872549]
2. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science.* 1993; 262:689–695. [PubMed: 7901908]
3. Choi DW. Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci.* 1995; 18:58–60. [PubMed: 7537408]
4. Sattler R, Tymiansky M. Molecular mechanisms of calcium-dependent excitotoxicity. *J Mol Med.* 2000; 78:3–13. [PubMed: 10759025]
5. Kip SN, et al. Changes in the expression of plasma membrane calcium extrusion systems during the maturation of hippocampal neurons. *Hippocampus.* 2006; 16:20–34. [PubMed: 16200642]
6. DeMarco SJ, Strehler EE. Plasma membrane Ca^{2+} -ATPase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family

of PDZ (PSD-95/Dlg/ZO-1) domain-containing proteins. *J Biol Chem.* 2001; 276:21594–21600. [PubMed: 11274188]

7. Yu L, Colvin RA. Regional differences in expression of transcripts for Na⁺/Ca²⁺ exchanger isoforms in rat brain. *Mol Brain Res.* 1997; 50:285–292. [PubMed: 9406945]
8. Li XF, Lytton J. Differential expression of Na/Ca exchanger and Na/Ca+K exchanger transcripts in rat brain. *Ann N Y Acad Sci.* 2002; 976:64–66. [PubMed: 12502535]
9. Papa M, et al. Differential expression of the Na⁺-Ca²⁺ exchanger transcripts and proteins in rat brain regions. *J Comp Neurol.* 2003; 461:31–48. [PubMed: 12722103]

FIGURE 1.

Effect of H₂O₂ on the expression of NCX transcripts in mature hippocampal neurons. RT-PCR was performed with NCX-specific primers using RNA from 21-day hippocampal neurons (HN), and from confluent glia-enriched cultures and total rat brain as positive control. The cells were either left untreated, or incubated for 2–3 hours in 300 μ M H₂O₂ (Glia+H₂O₂, HN+ H₂O₂) prior to RNA isolation. M, 100 bp marker ladder; C, negative control omitting template DNA. GAPDH internal controls are shown in the bottom panel.

FIGURE 2.

Effect of H_2O_2 on the expression and localization of PMCA in mature hippocampal neurons.

A: Hippocampal neurons cultured for 14 days were treated for 2 hours with 0, 150, and 300 μM H_2O_2 as indicated on top of each lane, and then harvested for SDS-polyacrylamide gel electrophoresis and Western blotting using antibody 5F10 against all PMCAs. The blot was also probed for β -actin as an internal protein loading control (bottom panel). The relative amount of PMCA standardized to the actin loading control is indicated beneath each lane. **B:** Immunofluorescence localization of PMCAs in 14-day hippocampal neurons left untreated (control, left panel), or incubated for 2 hours in the presence of 300 μM H_2O_2 (middle panel) or for 1 hour in 600 μM H_2O_2 (right panel).