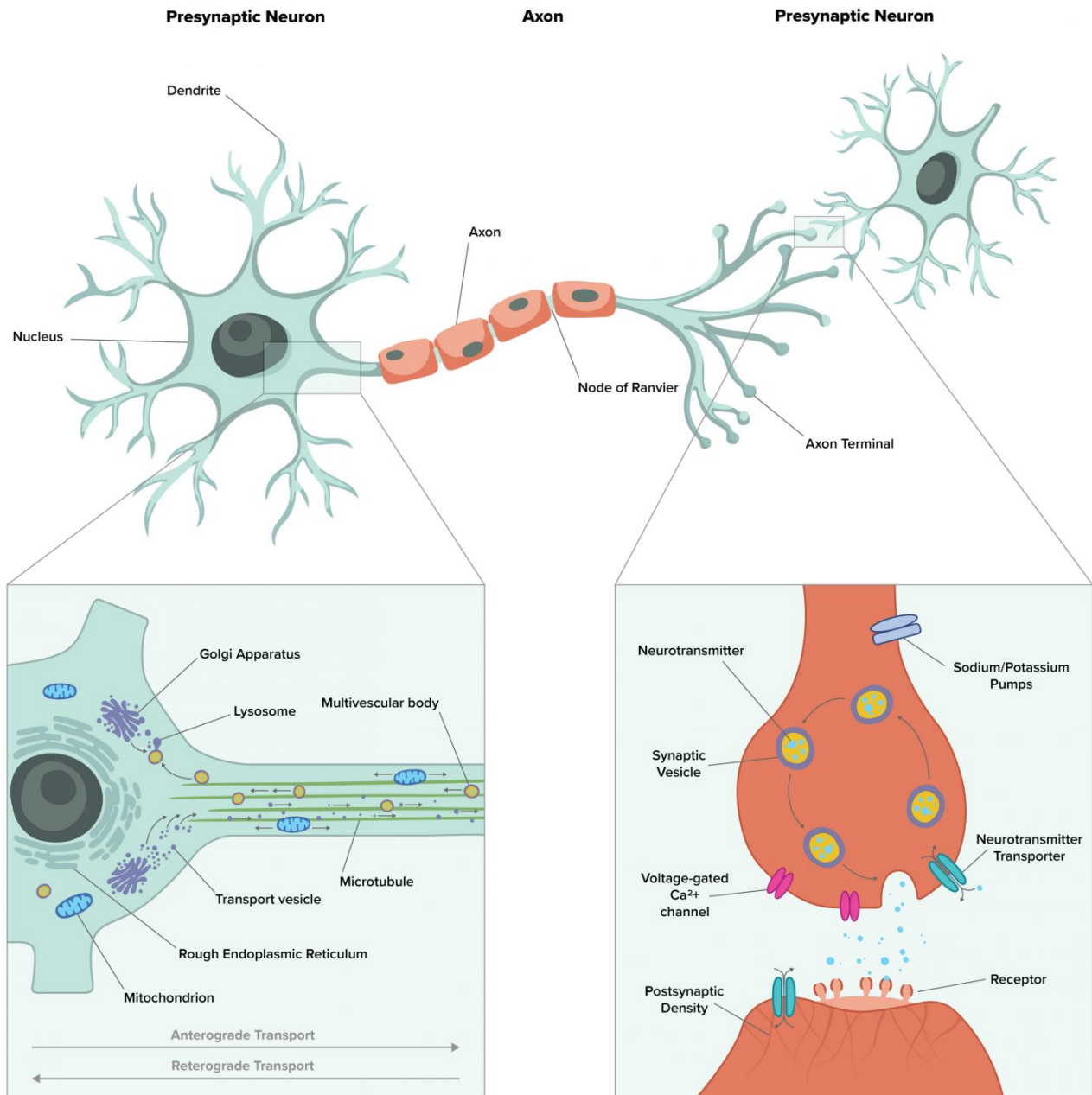


Contribution of oxidative stress to neurodegenerative disease

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High Energy Consuming Processes in Neurons: Axonal transport Synaptic vesicle recycling, Neurotransmitter release/reuptake, ion pumping, organelle maintenance, protein turnover.

Paul A. Hyslop, from Arkley BioTek Indianapolis, details an ongoing specific research approach to identify, characterize, and validate physiologically relevant neuronal targets of H₂O₂ in designing therapeutics for neurodegenerative disease progression

Can we modify neurodegenerative disease progression?

The societal impact of dementia resulting from the major neurodegenerative diseases such as Alzheimer's presents a growing and significant unmet medical need. ⁽¹⁾ Various cellular metabolic and structural deficits, neuroinflammation, and oxidative stress are known to result in the progressive loss of vulnerable non-regenerative neurons. ⁽²⁾

The continuously high metabolic demand of neurons for maintaining neuronal structural integrity and synaptic transmission, coupled with the sensitivity of neuronal energy metabolism to oxidative stress, underscores the importance of identifying metabolic components of energy metabolism that contribute to the initiation and progression of neurodegenerative disease. A successful research outcome will enable identifying and evaluating the safety and efficacy profiles of novel candidate therapeutics for the treatment of neurodegenerative disease in the clinic.

What are the origins of cellular H₂O₂?

Neuronal energy production by mitochondria depends on the coupling of glucose oxidation with reduction of molecular oxygen (O₂) to water, driving production of the cells major metabolic fuel (ATP). In healthy mitochondria, a small fraction of O₂ becomes partially reduced to superoxide, a precursor to H₂O₂ and other reactive oxygen species (ROS), as byproducts of aerobic metabolism. In a healthy neuron, the cellular antioxidant defense mechanisms adequately detoxify ROS, although constitutive production of H₂O₂ serves as an important cofactor for several metabolic transformations.

H₂O₂ is also produced by specialized enzymes in the cell membrane, particularly by cells of the innate immune system as part of host-defense against microbial and parasitic pathogens. ⁽³⁾ The term 'oxidative stress' describes conditions where excessive (pathological) H₂O₂ production by both mitochondria and inflammatory cells overwhelm the cells antioxidant systems, resulting in pathological tissue damage.

Correlation between H₂O₂ mediated oxidative stress and neurodegeneration

In a rat stroke model, analysis of H₂O₂ levels were measured in the dialysate from implanted microdialysis probes placed in the brain regions where substantial neuronal loss occurs following a period of ischemia and reperfusion. ⁽⁴⁾ Baseline levels of H₂O₂ were ~20 μM, rising to ~150 μM after initiation of a stroke followed by reperfusion. Histological examination of the neurons in the infarcted region of brain sections containing the dialysis probe demonstrated a severe loss of neurons.

In vitro, H₂O₂ concentrations of >150 μM H₂O₂ have repeatedly been demonstrated to be toxic to cortical and hippocampal neurons, showing a correlation between H₂O₂ oxidative stress and neurodegeneration and pathological levels of H₂O₂. The human brain consumes ~20% of the total oxygen demand of the body. ⁽⁵⁾

Interruption of oxygen and glucose supply to the brain for several minutes can result in neuronal dysfunction followed by neurodegeneration. These observations underscore the importance of glucose metabolism in maintaining intracellular ATP in neurons. A consistent finding is that in all cells in culture (including neurons), is that after exposure to H₂O₂, ATP levels decline.

Cellular targets of energy metabolism compromised by H₂O₂

Within the glycolytic pathway metabolizing glucose to pyruvate, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the only enzyme that is inactivated by H₂O₂.⁽⁶⁾ Inhibition of GAPDH was a significant contributor to loss of intracellular ATP. H₂O₂ also inhibited ATP production by mitochondria, and the total cellular loss of ATP was accounted for by inhibition of GAPDH activity and mitochondrial oxidative phosphorylation.⁽⁶⁾

The inhibition of GAPDH by H₂O₂ occurs by two distinct mechanisms. The major inactivation mechanism of the enzyme is by reversible S-glutathionylation of the H₂O₂ oxidized catalytic cysteine residue.⁽⁷⁾ At the same time, the minor inactivation mechanism involves a more complex oxidation of both the active site catalytic cysteine and also a vicinal downstream cysteine residue, irreversibly inactivating the enzyme.⁽⁸⁾

Metabolic consequences for GAPDH inactivation

S-glutathionylated GAPDH accumulates in neurons exposed to H₂O₂ and in tissues from patients with cardiovascular and neurodegenerative disease⁽⁹⁾, which is surprising given that deglutathionylation of intracellular proteins occurs by several dedicated processes. In vitro, S-glutathionylated GAPDH is rapidly deglutathionylated by low molecular weight thiols (such as 2-mercaptoethanol), resulting in full recovery of GAPDH activity.

In contrast, glutathione itself required much longer incubation times and higher concentrations to partially restore GAPDH enzyme activity. We explored reasons for this surprising observation in silico molecular modelling using the crystal structure of human GAPDH.⁽⁷⁾ We observed that S-glutathione was very tightly bound within the active site of GAPDH. This observation will likely explain (at least in part) why S-glutathionylated enzyme accumulates and underscores why H₂O₂ inhibits glycolysis, choking off substrate availability for mitochondrial ATP production. We also observed that S-glutathionylated GAPDH subunits undergo a significant reversible conformational rearrangement, discussed below.

Mechanisms of the reversible and irreversible GAPDH inactivation

Redox-active cysteine thiols are essential to many cellular metabolic transformations and the formation of the disulfide-linked cystine dimer for diversification of protein structure, function, and multi-subunit assembly. GAPDH is a tetramer, a group of four identical subunits each of which contains one active site, containing the essential catalytic cysteine residue of GAPDH (C152) that forms the acyl thioester intermediate of its substrates during the catalytic process.

H₂O₂ oxidizes C152 to a highly reactive cysteine sulfenic acid, which can then react with the major intracellular thiol pool of glutathione, forming reversible disulfide-linked S-glutathionylated cysteine adducts. Irreversible inactivation of GAPDH by H₂O₂ occurs by a unique consecutive oxidation mechanism ('two cysteine switch') first by oxidation of the enzyme's catalytic cysteine residue (C152) and second by oxidation of a cysteine residue four residues downstream (C156) within the active site region. of the enzyme, resulting in metastable conformational subunit rearrangement.⁽⁸⁾

We also observed that S-glutathionylation of C152 prevents subsequent oxidation of C156 by H₂O₂. As the reaction rate of C152 sulfenic acid with intracellular glutathione is at least an order of magnitude greater than that of C156 with H₂O₂, S-glutathionylated GAPDH is the predominant species observed in cells exposed to H₂O₂.

Conformational changes observed in oxidized GAPDH

Using in silico molecular modelling techniques, we have reported that both the H₂O₂ reversibly modified (S-glutathionylated enzyme) and the H₂O₂ irreversibly modified (C152/C156 oxidized enzyme) have conformational changes associated with a flexible sequence of amino acid residues known as the 'S-loop' region. However, the final structure of the two modified GAPDH subunits are significantly different in the two cases. This region of the GAPDH subunit is of interest because of its involvement in binding to other cellular proteins such as specific chaperones that direct the complexes to specific intracellular sites.

GAPDH, oxidative stress, and redox signaling

Several published research articles firmly established that following oxidative stress, both irreversibly oxidized GAPDH and S-glutathionylated GAPDH translocate to the nucleus, mediating cell signaling events that trigger cellular responses to the presence of oxidants. The most profound response is the induction of cell death, although there are many published examples of cell responses to oxidative stress involving GAPDH translocation following oxidative stress.

The divergent conformational changes that occur following the reversible and irreversible oxidation of GAPDH mediated by H₂O₂ indicate that future research efforts to understand the potential significance of this finding. The intersection of inhibition of energy metabolism and activation of redox signaling by GAPDH indicates an intimate link between these two processes.

Future therapeutics for neurodegenerative disease

Future research in this area should provide useful information regarding how neurons respond to oxidative stress in both physiological and pathophysiological settings and provide insights into identifying novel molecular targets for therapeutic intervention.

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