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Hydrogen Peroxide As a Diffusible Signal Molecule in Synaptic Plasticity

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Abstract

Reactive oxygen species (ROS) have been considered for some time only in the context of oxidative stress-induced cell damage. In this review, we discuss the growing body of evidence that implicates ROS in general, and hydrogen peroxide (H_2O_2) in particular, in regulatory events underlying synaptic plasticity. H_2O_2 is regarded in this context as a specific diffusible signaling molecule. The action of H_2O_2 is assumed to be carried out via the release of calcium ions from internal stores, modulating the activity of specific calcium-dependent protein phosphatases. These phosphatases eventually affect neuronal plasticity. We discuss the role of H_2O_2 in these systems, stressing the importance of cellular regulation of H_2O_2 levels that are altered in aging individuals, in the ability to express plasticity. These studies highlight the function of H_2O_2 in processes of learning and memory and their change in elderly individuals, irrespective of neurodegeneration found in Alzheimer's patients.

Index Entries: Synaptic plasticity; H_2O_2; aging; calcium; LTP; superoxide dismutase.

Introduction

A continuing rise in the incidence of age-associated neurodegenerative diseases has spurred extensive research in an attempt to discover their mechanisms, etiology, and prospects for therapy. A leading hypothesis proposes the involvement of reactive oxygen species (ROS), including superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) in neurodegenerative diseases. ROS are proposed to be involved in molecular processes leading to neurodegeneration through the adverse effects of oxidative stress—a condition in which more

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ROS are produced than the cellular defense mechanisms can handle, leading to eventual neuronal apoptosis. In this regard, the oxidative stress that induces apoptosis is believed to be the underlying mechanism of decline in neuronal efficacy. This mechanism has been proposed for Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) *(1),* which are diseases of the nervous system involving death of specific neurons, and an impairment of neurological systems in mostly aged patients. However, support for this hypothesis comes mostly from in vitro studies, many of which employ high concentrations of ROS that rarely exist in vivo *(2–4).* Nevertheless, and despite the inability to directly measure ROS reliably in living brains, there is a growing body of evidence indicating that aged brains are indeed exposed to higher concentrations of ROS than young ones *(5).* The cause for this rise remains unknown but a breakdown in mitochondrial regulation of intermediate oxidation products has been shown to occur in senescent individuals *(6).* Likewise, the cellular consequences of the putative rise in ROS are not clearly understood; which of the many molecular targets of ROS are the ones responsible for the loss of neuronal functions and how this loss is actually expressed *(7).*

We would like to propose a mechanism whereby reduced control over ROS production in aged brains could lead to impaired neuronal plasticity manifested as cognitive decline irrespective of, and prior to, ultimate cell death. We argue that the role of ROS is more complex than simple mediation of neuronal death, and we propose that ROS play a facilitatory action towards neuronal plasticity. Such a role has also been suggested recently *(8).* We review studies on bimodal action of ROS on neuronal properties, suggesting a role for H_2O_2 as a specific diffusible messenger molecule that modulates the activity of protein phosphatases, resulting in modulation of neuronal plasticity. Thus, when H_2O_2 levels are not under optimal regulation, cells may lose the ability to utilize H_2O_2 as a messenger molecule of neuronal plasticity, leading to changes in neuronal functions that could be expressed as cognitive or motor impairments before and irrespective of the eventual cell death evident in neurodegenerative diseases.

Reactive Oxygen Species

Since the beginning of aerobic life the high energy yield obtained by reducing oxygen atoms has served as a double-edged sword *(1).* Eukaryotic cells have quarantined these processes in the mitochondria utilizing these membranous organelles for creating a metabolically dependent proton gradient that could be utilized for producing ATP with superoxide anions as a byproduct. Although the mitochondria are probably the major source for superoxide anions, they can also be produced by NMDA receptor activation *(9),* which could be important for neuronal signaling. Superoxide anions contain an unpaired electron, which is highly reactive *(7,10).* If left unchecked, it can oxidize proton-rich molecules such as lipids, proteins, and nucleic acids, causing reduction in membrane fluidity, disturbance of cellular metabolism, or mutations, respectively. The enzyme superoxide dismutase (SOD) catalyses the reaction that converts superoxide to H_2O_2. H_2O_2 in itself is much less toxic than superoxide, however, it can be converted via the Fenton reaction in the presence of iron ions to hydroxyl radicals that are more reactive than superoxide (*see* Fig. 1). The in vivo occurrence of this reaction depends on the availability of free H_2O_2 and free iron *(1)* and has been regarded as the mechanism by which H_2O_2 can become toxic. H_2O_2 is normally converted to H_2O and O_2 by cellular antioxidants including catalase and glutathione peroxidase, however, under conditions termed "oxidative stress" more ROS are produced than can be handled and the overall redox state of a cell can be altered.

This view of ROS as agents of destruction wreaking havoc on lipids, proteins, and DNA promoted studies that use concentrations of ROS that are several orders of magnitude

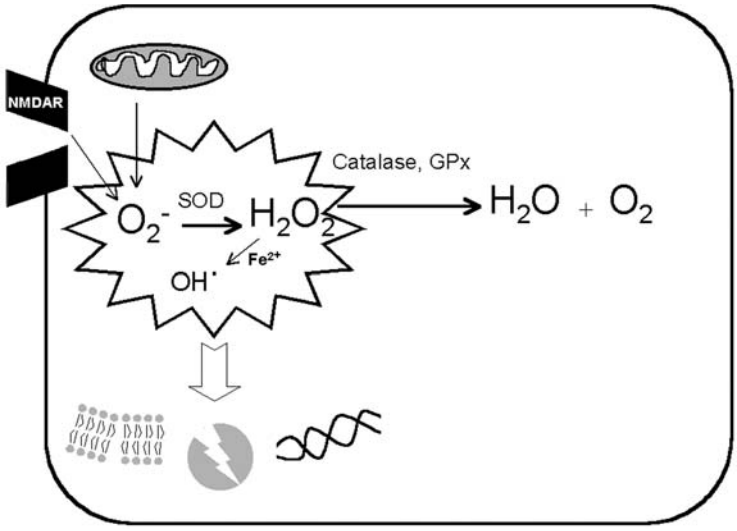

Fig. 1. Schematic view of cellular ROS management. Superoxide radicals are produced by mitochondria and NMDA receptors. This highly active radical can undergo dismutation by the enzyme SOD to form hydrogen peroxide, which in turn can form hydroxyl radicals via the Fenton reaction in the presence of free iron cations. These ROS can cause damage to lipids, proteins, and nucleic acids thus causing a disruption of cellular activities. The anti-oxidative enzymes catalase and glutathione peroxidase can facilitate the conversion of H_2O_2 to the benign water and oxygen molecules.

higher than those expected to be present in living cells, in an attempt to accelerate processes that are perceived to occur in vivo. But, what are the concentrations of ROS in vivo? A study by Hyslop et al. *(11),* found that the highest concentration of H_2O_2 in the striatum of a reperfused brain after an ischemic insult was estimated to be 100 μ*M.* In another microdialysis study, Lei et al. *(12)* determined basal H_2O_2 level in gerbil hippocampus to be about 1 μ*M.* These studies demonstrated a submillimolar concentration of H_2O_2 even under extreme acute pathological conditions that are known to generate ROS. Despite these low estimates there are virtually hundreds of studies showing that millimolar concentrations of H_2O_2 can produce apoptosis in different cell types including neurons (e.g., *2–4,13–16*). These studies aim at understanding mechanisms of neurodegeneration by studying oxidative stress induced apoptosis in different cell types. But what is the validity of these studies as models for neurodegeneration in the brain if the concentrations of H_2O_2 employed are at least 10–100 times higher than those assumed to be present in vivo? While it may be argued that the high concentrations of extracellular antioxidants present in the brain mask the true intracellular concentrations of ROS, this argument is not relevant to studies conducted with cell cultures that are grown in serum containing medium that is rich in these same antioxidants. Furthermore, neuronal apoptosis has not been proven to be the cause of the functional deficits seen in early stages of AD. In fact, early stages of AD and other neurodegenerative disorders are characterized by episodes of decline in neuronal functions followed by remission. A mechanism of neurodegeneration involving cell death, an irreversible process, would intuitively fail to explain these remissions. We must therefore search for mechanisms that affect the function of living neurons and not their viability. We assume that such mechanisms underlie neuronal plasticity.

Neuronal Plasticity

A cortical structure that has been employed extensively in the study of neurodegeneration is the hippocampus. The hippocampus is part of the temporal lobe, long known for its involvement in various forms of short-term memory processes in the brain. In humans, damaged hippocampus has been associated with loss of short-term memory, and these observations have been confirmed in numerous animal studies *(17).* The hippocampus undergoes massive degeneration in AD and as such has been a convenient target for many studies attempting to dissect the cellular and molecular mechanisms underlying age- and disease-related cell death, and their functional implications.

Long-term potentiation (LTP) and long-term depression (LTD), especially in the Schaffer collateral-CA1 synapses of hippocampal slices and in perforant path-dentate gyrus synapse in vivo are extensively studied models of synaptic plasticity. LTP is a long-lasting increase in synaptic efficacy following a potentiating event. Potentiating trains of pulses cause a persistent depolarization of postsynaptic membrane resulting in calcium ion flow through NMDA receptors and/or voltage-gated calcium channels (VGCCs) both in vivo and in vitro *(18,19).* This calcium flux results in well-characterized signal transduction cascades leading to a change in AMPA receptor permeability *(20,21)* which is manifested in larger (LTP) or smaller (LTD) excitatory postsynaptic potentials (EPSPs). This persistent change in EPSPs is regarded as a model for synaptic plasticity sharing mechanisms that are assumed to operate in learning and memory. Many of the transducing processes during and immediately after the potentiating stimulus are carried out by kinases and phosphatases (reviewed in ref. *22*).

Calcineurin Is a Key Participant in Neuronal Plasticity

One protein phosphatase that has been implicated in LTP is calcineurin, a calcium-dependent serine/threonine phosphatase that can dephosphorylate protein kinase A (PKA) substrates (such as calcium calmodulin kinase II, CamKII). Calcineurin can also activate protein phosphatase 1 (PP1) by dephosphorylating inhibitor-1. It has been hypothesized *(23)* that this route is active in calcineurin inhibition of pCREB activation in response to depolarizing stimuli in hippocampal neurons. Enhanced activity of calcineurin in a genetic model *(24)* caused a decrease in some forms of LTP, whereas inhibiting brain calcineurin in another genetic model enhanced memory and LTP *(25).* Interestingly, forebrain-specific calcineurin knockout resulted mostly in impaired LTD and working memory *(26).* Inhibiting calcineurin activity in CA1 cells caused potentiation of EPSPs in slices from adult but not young rats *(27),* while in another study Onuma et al. *(28)* show that blocking calcineurin inhibited VGCC LTP in CA1 of 7–10 wk old mice. These studies establish a role for calcineurin in synaptic plasticity with a variety in the direction of this effect (enhancing or inhibiting LTP) depending on the background conditions. Interestingly, a calcineurin inhibitory gene *DSCR1* is expressed in brains of AD patients three fold over that of controls. The expression of this gene could be induced by the amyloid β 1-42 peptide associated with senile plaques *(29);* this may indicate pathologically low calcineurin activity in AD brains as a contributing factor in mental decline. Conversely, it may indicate a response to pathologically high activity of calcineurin, which may be induced by ROS.

Calcineurin and Age-Dependent Hippocampal Decline

Calcineurin is a good candidate for linking age-dependent deficiencies in hippocampal functions and calcium-dependant cellular mechanisms, as suggested by Foster et al. *(30).* They have shown an elevation in calcineurin activity as well as in the activity of calcineurin-regulated PP1 in aged rats that

were impaired in hippocampus dependent memory tasks.

Calcineurin can be activated by H_2O_2. Nuclear factor of activated T cells (NFAT) is a transcription factor that is activated by calcineurin. NFAT activity can be induced by asbestos *(31)* or vanadium *(32)* in a manner that is dependent on H_2O_2 production. It can be enhanced by the H_2O_2 producing enzyme superoxide dismutase (SOD) and can be blocked by the calcineurin inhibitor Cyclosporin A. Inactivation of calcineurin by H_2O_2 has been shown *(33)*, however this study used 1 m*M* H_2O_2 for time dependence of the reaction showing 75% activity decrease after 30 min. And only as little as 0.3 m*M* H_2O_2 for dose dependence, under which an activity plot was extrapolated. Accordingly, activation of calcineurin by micromolar concentrations of H_2O_2 cannot be ruled out in that study *(33)*.

CamKII activates calcineurin after being exposed to a rise in intracellular calcium. Several studies have shown that H_2O_2 can induce an increase in intracellular calcium using various cell types and 0.01–10 m*M* of H_2O_2 *(16,34–41)*. In some of these studies the increase in intracellular calcium was sensitive to thapsigargin indicating its origin in intracellular calcium stores. These studies show that a change in ROS can induce a change in calcium, which can then have an effect on calcineurin.

ROS in Brain Plasticity Studies

As mentioned above, aging of the brain is accompanied by an increase in ROS production. Concomitantly, hippocampal slices taken from aged and young adult rats exhibit different responses to similar trains of stimulation. Slices from aged rats are impaired in LTP and they exhibit LTD in response to frequencies of stimulation that do not affect young rats. Nifedipine, an L-type VGCC blocker can reverse some of these changes *(42)*. O'Donnell et al. *(5)* also reported an age-dependent decrease in LTP in rats; a decline thought to be due to oxidative stress. They also demonstrated an age-dependent increase in SOD activity. Moreover, stress activated genes that were upregulated in aged individuals could be activated in synaptosomes by applying H_2O_2 (albeit at a high concentration of 5 m*M*). An interesting study by Vereker et al. *(43)* demonstrated a connection between stress, SOD activity, and LTP decline. Stress induced a decrease in LTP, an increase in SOD activity and an increase in IL-1β concentration. An increase in IL-1β concentration could increase SOD activity without the stress. IL-1β alone also inhibited LTP in a manner that was reversible by adding anti-oxidants, demonstrating that the IL-1β mediated stress induced LTP decline was associated with a rise in ROS. Moreover, they showed a similar decline in LTP in the presence of 200 μ*M* H_2O_2. Interestingly, dietary manipulation with anti-oxidants could restore LTP in aged individuals that were otherwise impaired. McGahon et al. *(44)*, have demonstrated a reversal of age-dependent LTP decline by dietary supplementation with omega-3 fatty acids as did Liu et al. *(45)* by feeding other anti-oxidative fatty acids: acetyl-L-carnitine and/or R-α-lipoic acid.

Direct Application of H_2O_2 to Brain Slices

Several groups have applied H_2O_2 to brain slices for the purpose of studying the effects of ROS on synaptic plasticity. Pellmar et al. *(46)* have shown that 0.5 m*M* H_2O_2 can inhibit long term potentiation (LTP) in guinea pig hippocampal slices. Avshalumov et al. *(47)* showed a dramatic effect of 1.2–2 m*M* H_2O_2 in reducing population spikes in hippocampal slices with a consequent epileptiform activity when H_2O_2 was washed out *(48)*. These concentrations of H_2O_2 are likely to produce nonspecific effects, with respect to LTP induction mechanisms. In contrast, some of the LTP-related phenomena seen in aged animals could be mimicked in younger animals exposed to physiologically relevant concentrations of H_2O_2; a low concentration of H_2O_2 (29 μ*M*)

inhibits muscarinic as well as tetanically induced LTP in hippocampal slices *(49).*

We have shown recently *(8),* that applying 20 μ*M* of H_2O_2 to hippocampal slices taken from rats resulted in reduction of LTP and an increase in LTD without affecting baseline properties. Blocking calcineurin could antagonize this effect of H_2O_2. Calcineurin was also shown to be more active in hippocampal slices exposed to 20 μ*M* H_2O_2. Interestingly, 1 μ*M* of H_2O_2 had a reverse effect, enhancing LTP to double that of control. This effect of H_2O_2 was also blocked by calcineurin inhibitors but also by rapamycin—an inhibitor of FKBP-12, which does not interact with calcineurin. When bound by rapamycin, FKBP-12 dissociates from ryanodine receptors that control calcium flow from internal stores. H_2O_2 at a concentration of 1 μ*M* also decreased the range of low stimulation frequencies that could elicit LTD. These studies demonstrate the effect of H_2O_2 on synaptic plasticity when it is applied concurrently with trains of stimulation, which normally elicit synaptic plasticity. However, aged individuals are exposed to chronically altered levels of ROS, which may affect synaptic plasticity in different ways.

Transgenic SOD Overexpressing Mice

Under normal conditions, superoxide radicals produced by the mitochondria but also by the activity of ion channels, are scavenged by the enzyme SOD. SOD converts superoxide to H_2O_2, which is a less reactive, membrane permeable intermediate. Interestingly, the gene encoding the SOD message resides in humans on chromosome 21 in a region that is triplicated in Down syndrome—a genetic form of mental retardation that shares pathological hallmarks with AD. Transgenic mice overexpressing human SOD (tg-SOD) were generated *(50)* and have been extensively studied as tentative models of neurodegeneration. These mice express several copies of SOD and the brains of the transgenic strain we are currently using show sixfold enhanced activity of the enzyme over controls. The neuromuscular junction in the tongue of tg-SOD mice is degenerated *(51)* and they also exhibit thymic abnormalities *(52).* On the other hand, tg-SOD mice were found to be less susceptible to focal cerebral ischemic injury *(53,54)* and SOD overexpressing rats were also protected against ischemia *(55).* While kainic acid induced apoptosis is exacerbated in cultured neurons from tg-SOD mice *(52),* whole animals injected with kainic acid are protected from seizure in comparison to controls *(56).* Thymocytes from tg-SOD mice have been shown to produce more H_2O_2 than controls *(52).* We have shown *(57)* that transgenic mice overexpressing SOD were impaired in spatial memory tasks. Hippocampal slices taken from these mice were impaired in LTP in a manner that was reversed by catalase, an enzyme that breaks down H_2O_2, and also by the spin trapping agent *N-t*-butyl-phenylnitrone. We also found impairment in perforant path LTP measured in vivo in SOD transgenic mice *(56),* as well as resistance to kainic acid which induced seizures in wild-type controls. Hippocampal cells in the transgenic mice were under a high level of GABAergic inhibition manifested in over-activity of interneurons in slices from SOD mice. Consequently, bicuculline, a GABAergic antagonist, could restore LTP in the dentate gyrus of SOD mice.

In our recent studies conducted with these mice *(58)* we were able to restore LTP in hippocampal slices of tg-SOD mice by perfusing them with 50 μ*M* H_2O_2 in a calcineurin-dependent manner. This concentration of H_2O_2 inhibited LTP in the wt controls. We were intrigued to find that aged (2 yr old) tg-SOD slices had altered calcineurin activity and larger LTP than controls. Aged wt slices were impaired in LTP in a manner reversible by 50 μ*M* H_2O_2, as was the case with young tg-SOD mice, and they also had high levels of endogenous ROS and phosphatase activity.

Interestingly, mutations leading to the neurodegenerative disease ALS have been mapped to the SOD gene, however it is believed that these mutations do not inhibit the dismutase activity, rather, they constitute a "gain-of-func-

tion" which may also lead to oxidative stress through a decrease in the enzymes affinity to zinc *(59)*.

It has been suggested that the LTP impairment as well as memory deficiencies seen in tg-SOD mice are due to a rapid elimination of superoxide radicals produced by the activation of NMDA receptors and not as a consequence of high levels of H_2O_2. This can be deduced from the elimination of LTP with the addition of superoxide scavengers *(60,61)* and is supported by the fact that catalase added to hippocampal slices exposed to SOD as well as to slices from extracellular SOD overexpressing mice *(62)*, could not restore LTP. These results were interpreted as an indication that the overproduction of H_2O_2 by itself is not the cause for the reduction in efficacy of LTP, but perhaps the fast removal of superoxides by SOD. While this is an interesting interpretation of the data, the biochemical nomenclature of unit definitions of different enzymes is such that enzymatic activity of different enzymes cannot be compared on a scale of units. Therefore, 121 U/mL of SOD do not necessarily produce less H_2O_2 than 260 units/mL of catalase can break down. Furthermore, externally perfused catalase would have to permeate the slice and be active at the synaptic cleft, and with negative results, and lack of independent confirmation that the enzyme did work, such data is hard to interpret. These studies further support the involvement of ROS in the regulation of synaptic plasticity.

In the same study, bicuculline was added to hippocampal slices and LTP was not restored. We have found the same with slices from intracellular SOD overexpressing mice (*see* Fig. 2). This apparent discrepancy with our perforant path in vivo study may result from the differences between the preparations, specifically, the low amount of interneuron inhibitory innervation that results from transforming a three-dimensional hippocampus to a two-dimensional slice.

It has been hypothesized that superoxide can activate protein kinases and inhibit protein phosphatases, such as calcineurin *(63)*, however

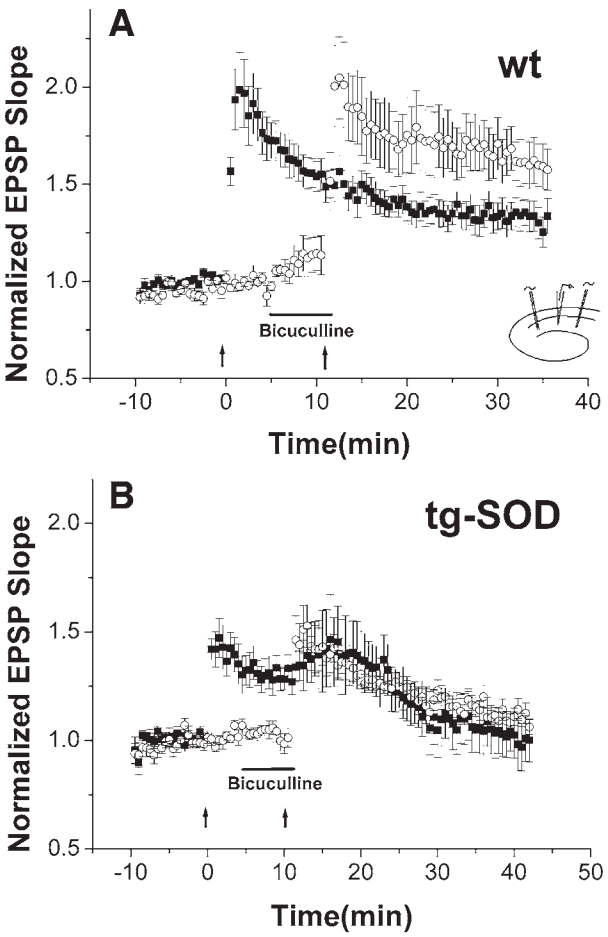

Fig. 2. LTP in hippocampal slices from wild type (wt) and transgenic SOD overexpressing (tg-SOD) mice. Hippocampal slices were prepared as described elsewhere *(8)* and placed in a recording chamber with a single recording electrode in CA1 stratum radiatum and two stimulating electrodes as shown schematically. **(A)** Wt slices were stimulated by applying theta burst stimulation (TBS, arrow) through one electrode (squares) which resulted in excitatory postsynaptic potentials (EPSPs) that were 1.38 ± 0.07 of baseline 25 min after TBS. This was followed by application of 10 μ*M* bicuculline (bar) for 5 min followed by TBS through the second electrode (circles) resulting in EPSPs 1.59 ± 0.11 25 min after TBS. **(B)** Tg-SOD mice are impaired in LTP which cannot be rescued by bicuculline. EPSPs of the first channel 20 min after bicuculine were 1.04 ± 0.1 of baseline, and EPSPs of the second channel were 1.15 ± 0.04 of baseline 20 min after TBS.

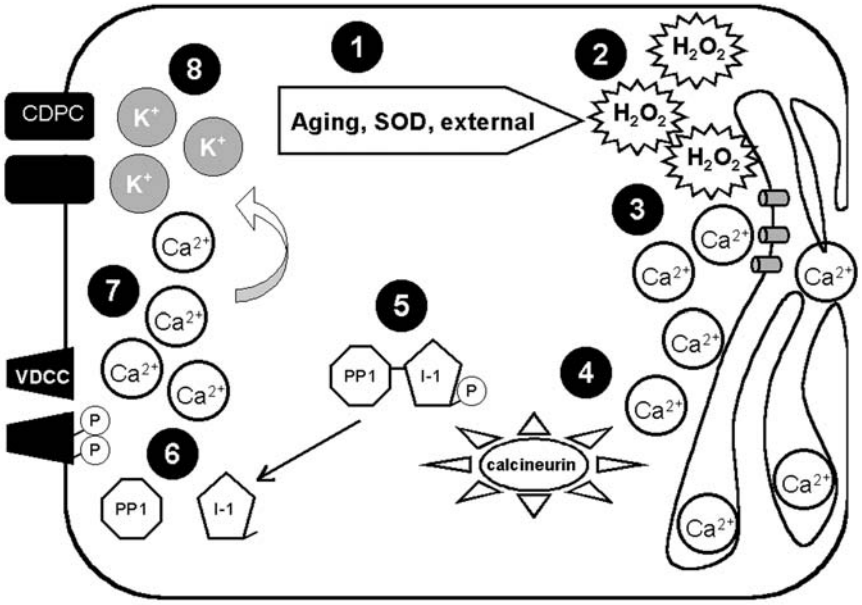

Fig. 3. Representation of the sequence of events that may link between redox changes and alterations in synaptic plasticity. Aging, transgenic intervention, or exogenous addition of H_2O_2 (1) can increase the intracellular concentration of H_2O_2 (2) which can then cause the release of calcium from internal stores (3), activating calcineurin (4); calcineurin-mediated dephosphorylation of Inhibitor-1 (5) allows protein phosphatase 1 to dephosphorylate PKA substrates on VGCCs (6), altering the permeability of these to calcium (7) which may alter the opening time of calcium-dependant potassium channels (8) leading to a change in synaptic plasticity.

most of the evidence suggesting phosphatase inhibition stems from experiments using high levels of oxidants. Interestingly, Knapp and Klann *(64)* have demonstrated that LTP could be induced in rat hippocampal slices via a PKC dependent pathway by generating superoxide radicals with the xanthine/ xanthine oxidase system.

The age-dependent effect of transgenically overexpressed SOD on LTP provides a link between chronically elevated levels of H_2O_2 and synaptic plasticity deficiencies similar to those seen in aged individuals.

H_2O_2 and Calcium Channels

Aging, SOD overexpression, and direct application of H_2O_2, can increase the tissue level of H_2O_2. Elevated H_2O_2 can induce the release of calcium from intracellular stores and this excess calcium can induce calcineurin activity via CamKII. Active calcineurin dephosphorylates I-1 resulting in higher activity of PP1 (*see* Fig. 3). Which substrate of PP1 could induce changes in LTP?

Norris et al. *(42)* found that blocking PP1 and PP2A enhanced EPSPs in aged but not in adult rats whereas H-7, a serine / threonine kinase inhibitor decreased EPSPs in adult but not aged rats. Blocking potassium currents with apamin was also effective in restoring LTP in aged slices *(65)*, suggesting a role for calcium-dependent potassium channels in age-dependent LTP decline. These data are compatible with Campbell et al. *(66)* who measured an age-dependent increase in L-type VGCC current. Furthermore, Mermelstein et al. *(67)* have shown that the state of activity of L-type VGCCs can differentially regulate signal transduction cascades.

Nifedipine, an L-type VGCC blocker was effective in reversing the effects of 1μ*M* H_2O_2 on LTP *(8).* Recently, Norris et al. *(68)* have shown that specific blockade of calcineurin decreased VGCC currents in cultured hippocampal neurons in a manner that was increased in cultures that were 4 wk old over cultures that were 2 wk old. VGCC permeability can be controlled by PKA phosphorylation of several sites on the channel protein *(69,70).*

A calcium imaging study conducted in hippocampal slices taken from young adult and aged rats measured responses to 7 Hz stimulation: aged slices were found to be impaired in frequency facilitation in a manner that could be mimicked in young slices by adding the VGCC agonist Bay K8644 *(71)* demonstrating an age-dependent change in VGCC permeability. When postsynaptic cells are depolarized by sustained stimulation, VGCCs remain open long enough to allow an inward calcium current that acts as a messenger for cellular events. One of the targets of this calcium current is a calcium-dependent potassium channel that affects the cells ability to undergo further depolarization. Changing the calcium permeability of VGCCs by H_2O_2 can thus change the message that a postsynaptic cell is receiving.

Conclusions

Physiologically relevant concentrations of H_2O_2, within the 1–50 μ*M* range can induce changes in synaptic plasticity. These concentrations of H_2O_2 have been shown to release calcium from intracellular stores. The release of calcium may result from a redox sensitive domain on proteins controlling this release such as ryanodine receptors. Once a shift in redox state has occurred, either by external addition of ROS or by genetic manipulation of cellular anti-oxidants or by normal aging, a change in calcium may follow. This excess calcium can activate calcium-dependent proteins such as CamKII, calcineurin and PP1, which in turn can alter the calcium permeability of L-type VGCCs. An altered calcium flux at the time of synaptic potentiating events can change the neuronal meaning of that event by inducing changes in calcium-dependent potassium currents (Fig. 3). While our data make a strong case for calcineurin as the main transducer of H_2O_2-mediated signaling in hippocampal slices, this signaling may involve other, as yet undiscovered transduction cascades. We do, however, mean to emphasize the perception of H_2O_2 as an important signaling molecule. H_2O_2 is a short-lived, membrane permeable, oxidant that is well suited for the role of messenger. This messenger can induce the release of calcium on both sides of the synapse triggering concerted activity. Accordingly, H_2O_2 acting as an acute messenger molecule produced by the activity of ion channels depends on existing levels of H_2O_2 prior to generation of plastic events. A high background level of H_2O_2 can induce higher activity of anti-oxidants, it can also alter the redox sensitivity of target molecules. In this way, a high ambient H_2O_2 level will dampen the effect of an H_2O_2 flux that results from synaptic activity. Thus, our model explains some of the age-related neuronal phenomena and allows for testable predictions that may ultimately improve the way we treat elderly patients with neurodegenerative diseases.

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References

1 Halliwell B. (1992) Reactive oxygen species and the central nervous system. *J. Neurochem.* **59,** 1609–1623.

2 Kanno S, Ishikawa M, Takayanagi M, Takayanagi Y, and Sasaki K. (1999) Exposure to hydrogen peroxide induces cell death via apoptosis in primary cultured mouse hepatocytes. *Biol. Pharm. Bull.* **22,** 1296–1300.

3 Burlacu A, Jinga V, Gafencu AV, and Simionescu M. (2001) Severity of oxidative stress generates

different mechanisms of endothelial cell death. *Cell Tissue Res.* **306,** 409–416.
4 Datta K, Babbar P, Srivastava T, Sinha S, and Chattopadhyay P. (2002) p53 dependent apoptosis in glioma cell lines in response to hydrogen peroxide induced oxidative stress. *Int. J. Biochem. Cell Biol.* **34,** 148–157.
5. O'Donnell E, Vereker E, and Lynch MA. (2000) Age-related impairment in LTP is accompanied by enhanced activity of stress-activated protein kinases: analysis of underlying mechanisms. *Eur. J. Neurosci.* **12,** 345–352.
6 Lopez-Torres M, Gredilla R, Sanz A, and Barja G. (2002) Influence of aging and long-term caloric restriction on oxygen radical generation and oxidative DNA damage in rat liver mitochondria. *Free Radic. Biol. Med.* **32,** 882–889.
7 Beckman KB and Ames BN. (1998) The free radical theory of aging matures. *Physiol. Rev.* **78,** 547–581.
8 Kamsler A and Segal M. (2003) Hydrogen peroxide modulation of synaptic plasticity. *J. Neurosci.* **23,** 269–276.
9 Lafon-Cazal M, Pietri S, Culcasi M, and Bockaert J. (1993) NMDA-dependent superoxide production and neurotoxicity. *Nature* **364,** 535–537.
10 Benzi G and Moretti A. (1995) Are reactive oxygen species involved in Alzheimer's disease? *Neurobiol. Aging.* **16,** 661–674.
11 Hyslop PA, Zhang Z, Pearson DV, and Phebus LA. (1995) Measurement of striatal H_2O_2 by microdialysis following global forebrain ischemia and reperfusion in the rat: correlation with the cytotoxic potential of H_2O_2 in vitro. *Brain Res.* **671,** 181–186.
12 Lei B, Adachi N, and Arai T. (1998) Measurement of the extracellular H_2O_2 in the brain by microdialysis. *Brain Res. Brain Res. Protoc.* **3,** 33–36.
13 Jang JH and Surh YJ. (2001) Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells. *Mutat. Res.* **496,** 181–190.
14 Crossthwaite AJ, Hasan S, and Williams RJ. (2002) Hydrogen peroxide-mediated phosphorylation of ERK1/2, Akt/PKB and JNK in cortical neurones: dependence on Ca(2+) and PI3-kinase. *J. Neurochem.* **80,** 24–35.
15 Bhat NR and Zhang P. (1999) Hydrogen peroxide activation of multiple mitogen-activated protein kinases in an oligodendrocyte cell line: role of extracellular signal-regulated kinase in hydrogen peroxide-induced cell death. *J. Neurochem.* **72,** 112–119.
16 Herson PS, Lee K, Pinnock RD, Hughes J, and Ashford ML. (1999) Hydrogen peroxide induces intracellular calcium overload by activation of a non-selective cation channel in an insulin-secreting cell line. *J. Biol. Chem.* **274,** 833–841.
17 Manns JR, Hopkins RO, and Squire LR. (2003) Semantic memory and the human hippocampus. *Neuron.* **38,** 127–133.
18 Morgan SL and Teyler TJ. (1999) VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. *J. Neurophysiol.* **82,** 736–740.
19 Borroni AM, Fichtenholtz H, Woodside BL, and Teyler TJ. (2000) Role of voltage-dependent calcium channel long-term potentiation (LTP) and NMDA LTP in spatial memory. *J. Neurosci.* **20,** 9272–9276.
20 Bliss TV and Collingridge GL. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361,** 31–39.
21 Dudek SM and Bear MF. (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proc. Natl. Acad. Sci. USA* **89,** 4363–4367.
22 Soderling TR and Derkach VA. (2000) Postsynaptic protein phosphorylation and LTP. *Trends Neurosci.* **23,** 75–80.
23 Bito H, Deisseroth K, and Tsien RW. (1996) CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* **87,** 1203–1214.
24 Winder DG, Mansuy IM, Osman M, Moallem TM, and Kandel ER. (1998) Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* **92,** 25–37.
25 Malleret G, Haditsch U, Genoux D, et al. (2001) Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell* **104,** 675–686.
26 Zeng H, Chattarji S, Barbarosie M, et al. (2001) Forebrain-specific calcineurin knockout selectively impairs bidirectional synaptic plasticity and working/episodic-like memory. *Cell* **107,** 617–629.
27 Wang JH and Kelly PT. (1997) Postsynaptic calcineurin activity downregulates synaptic transmission by weakening intracellular Ca^{2+} signaling mechanisms in hippocampal CA1 neurons. *J. Neurosci.* **17,** 4600–4611.
28 Onuma H, Lu YF, Tomizawa K, Moriwaki A, Tokuda M, Hatase O, and Matsui H. (1998) A

calcineurin inhibitor, FK506, blocks voltage-gated calcium channel-dependent LTP in the hippocampus. *Neurosci. Res.* **30,** 313–319.
29. Ermak G, Morgan TE, and Davies KJ. (2001) Chronic overexpression of the calcineurin inhibitory gene DSCR1 (Adapt78) is associated with Alzheimer's disease. *J. Biol. Chem.* **276,** 38,787–38,794.
30. Foster TC, Sharrow KM, Masse JR, Norris CM, and Kumar A. (2001) Calcineurin links Ca^{2+} dysregulation with brain aging. *J. Neurosci.* **21,** 4066–4073.
31. Li J, Huang B, Shi X, Castranova V, Vallyathan V, and Huang C. (2002) Involvement of hydrogen peroxide in asbestos-induced NFAT activation. *Mol. Cell Biochem.* **234,** 161–168.
32. Huang C, Ding M, Li J, et al. (2001) Vanadium-induced nuclear factor of activated T cells activation through hydrogen peroxide. *J. Biol. Chem.* **276,** 22,397–22,403.
33. Bogumil R, Namgaladze D, Schaarschmidt D, Schmachtel T, Hellstern S, Mutzel R, and Ullrich V. (2000) Inactivation of calcineurin by hydrogen peroxide and phenylarsine oxide. Evidence for a dithiol-disulfide equilibrium and implications for redox regulation. *Eur. J. Biochem.* **267,** 1407–1415.
34. Roveri A, Coassin M, Maiorino M, Zamburlini A, van Amsterdam FT, Ratti E, and Ursini F. (1992) Effect of hydrogen peroxide on calcium homeostasis in smooth muscle cells. *Arch. Biochem. Biophys.* **297,** 265–270.
35. Volk T, Hensel M, and Kox WJ. (1997) Transient Ca^{2+} changes in endothelial cells induced by low doses of reactive oxygen species: role of hydrogen peroxide. *Mol. Cell Biochem.* **171,** 11–21.
36. Nakazaki M, Kakei M, Yaekura K, Koriyama N, Morimitsu S, Ichinari K, Yada T, and Tei C. (2000) Diverse effects of hydrogen peroxide on cytosolic Ca^{2+} homeostasis in rat pancreatic beta-cells. *Cell Struct. Funct.* **25,** 187–193.
37. Lee ZW, Kweon SM, Kim BC, Leem SH, Shin I, Kim JH, and Ha KS. (1998) Phosphatidic acid-induced elevation of intracellular Ca^{2+} is mediated by RhoA and H_2O_2 in Rat-2 fibroblasts. *J. Biol. Chem.* **273,** 12,710–12,715.
38. Yermolaieva O, Brot N, Weissbach H, and Heinemann SH, and Hoshi T. (2000) Reactive oxygen species and nitric oxide mediate plasticity of neuronal calcium signaling. *Proc. Natl. Acad. Sci. USA* **97,** 448–453.
39. Yang ZW, Zheng T, Wang J, Zhang A, Altura BT, and Altura BM. (1999) Hydrogen peroxide induces contraction and raises $[Ca^{2+}]i$ in canine cerebral arterial smooth muscle: participation of cellular signaling pathways. *Naunyn Schmiedebergs Arch. Pharmacol.* **360,** 646–653.
40. Nam SH, Jung SY, Yoo CM, Ahn EH, and Suh CK. (2002) H_2O_2 enhances Ca^{2+} release from osteoblast internal stores. *Yonsei Med. J.* **43,** 229–235.
41. Gen W, Tani M, Takeshita J, Ebihara Y, and Tamaki K. (2001) Mechanisms of Ca^{2+} overload induced by extracellular H_2O_2 in quiescent isolated rat cardiomyocytes. *Basic Res. Cardiol.* **96,** 623–629.
42. Norris CM, Halpain S, and Foster TC. (1998) Alterations in the balance of protein kinase/phosphatase activities parallel reduced synaptic strength during aging. *J. Neurophysiol.* **80,** 1567–1570.
43. Vereker E, O'Donnell E, Lynch A, Kelly A, Nolan Y, and Lynch MA. (2001) Evidence that interleukin-1beta and reactive oxygen species production play a pivotal role in stress-induced impairment of LTP in the rat dentate gyrus. *Eur. J. Neurosci.* **14,** 1809–1819.
44. McGahon BM, Martin DS, Horrobin DF, and Lynch MA. (1999) Age-related changes in synaptic function: analysis of the effect of dietary supplementation with omega-3 fatty acids. *Neuroscience* **94,** 305–314.
45. Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, Cotman CW, and Ames BN. (2002) Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-alpha-lipoic acid. *Proc. Natl. Acad. Sci. USA* **99,** 2356–2361.
46. Pellmar TC, Hollinden GE, and Sarvey JM. (1991) Free radicals accelerate the decay of long-term potentiation in field CA1 of guinea-pig hippocampus. *Neuroscience* **44,** 353–359.
47. Avshalumov MV, Chen BT, and Rice ME. (2000) Mechanisms underlying H(2)O(2)-mediated inhibition of synaptic transmission in rat hippocampal slices. *Brain Res.* **882,** 86–94.
48. Avshalumov MV and Rice ME. (2002) NMDA receptor activation mediates hydrogen peroxide induced pathophysiology in rat hippocampal slices. *J. Neurophysiol.* **87,** 2896–2903.
49. Auerbach JM and Segal M. (1997) Peroxide modulation of slow onset potentiation in rat hippocampus. *J. Neurosci.* **17,** 8695–8701.
50. Epstein CJ, Avraham KB, Lovett M, Smith S, Elroy-Stein O, Rotman G, Bry C, and Groner Y. (1987) Transgenic mice with increased Cu/Zn-superoxide dismutase activity: animal model of

dosage effects in Down syndrome. *Proc. Natl. Acad. Sci. USA* **84,** 8044–8048.
51. Yarom R, Sapoznikov D, Havivi Y, Avraham KB, Schickler M, and Groner Y. (1988) Premature aging changes in neuromuscular junctions of transgenic mice with an extra human CuZn-SOD gene: a model for tongue pathology in Down's syndrome. *J. Neurol. Sci.* **88,** 41–53.
52. Peled-Kamar M, Lotem J, Okon E, Sachs L, and Groner Y. (1995) Thymic abnormalities and enhanced apoptosis of thymocytes and bone marrow cells in transgenic mice overexpressing Cu/Zn-superoxide dismutase: implications for Down syndrome. *EMBO J.* **14,** 4985–4993.
53. Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, and Chan PH. (1991) Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. *Proc. Natl. Acad. Sci. USA* **88,** 11,158–11,162.
54. Saito A, Hayashi T, Okuno S, Ferrand-Drake M, and Chan PH. (2003) Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the Bad cell death signaling pathway. *J. Neurosci.* **23,** 1710–1718.
55. Sugawara T, Noshita N, Lewen A, et al. (2002) Overexpression of copper/zinc superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. *J. Neurosci.* **22,** 209–217.
56. Levkovitz Y, Avignone E, Groner Y, and Segal M. (1999) Upregulation of GABA neurotransmission suppresses hippocampal excitability and prevents long-term potentiation in transgenic superoxide dismutase-overexpressing mice. *Neurosci.* **19,** 10,977–10,984.
57. Gahtan E, Auerbach JM, Groner Y, and Segal M. (1998) Reversible impairment of long-term potentiation in transgenic Cu/Zn-SOD mice. *Eur. J. Neurosci.* **10,** 538–544.
58. Kamsler A, Segal M. (2003) Paradoxical actions of hydrogen peroxide on LTP in transgenic SOD-1 mice. *J. Neurosci.* **23,** 10359–10367.
59. Beckman JS, Estevez AG, Crow JP, and Barbeito L. (2001) Superoxide dismutase and the death of motoneurons in ALS. *Trends Neurosci.* **24,** S15–20.
60. Klann E. (1998) Cell-permeable scavengers of superoxide prevent long-term potentiation in hippocampal area CA1. *J. Neurophysiol.* **80,** 452–457.
61. Klann E, Roberson ED, Knapp LT, and Sweatt JD. (1998) A role for superoxide in protein kinase C activation and induction of long-term potentiation. *J. Biol. Chem.* **273,** 4516–4522.
62. Thiels E, Urban NN, Gonzalez-Burgos GR, et al. (2000) Impairment of long-term potentiation and associative memory in mice that overexpress extracellular superoxide dismutase. *J. Neurosci.* **20,** 7631–7639.
63. Klann E and Thiels E. (1999) Modulation of protein kinases and protein phosphatases by reactive oxygen species: implications for hippocampal synaptic plasticity. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **23,** 359–376.
64. Knapp LT and Klann E. (2002) Potentiation of hippocampal synaptic transmission by superoxide requires the oxidative activation of protein kinase C. *J. Neurosci.* **22,** 674–683.
65. Norris CM, Halpain S, and Foster TC. (1998) Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca^{2+} channels. *J. Neurosci.* **18,** 3171–3179.
66. Campbell LW, Hao SY, Thibault O, Blalock EM, and Landfield PW. (1996) Aging changes in voltage-gated calcium currents in hippocampal CA1 neurons. *J. Neurosci.* **16,** 6286–6295.
67. Mermelstein PG, Bito H, Deisseroth K, and Tsien RW. (2000) Critical dependence of cAMP response element-binding protein phosphorylation on L-type calcium channels supports a selective response to EPSPs in preference to action potentials. *J. Neurosci.* **20,** 266–273.
68. Norris CM, Blalock EM, Chen KC, Porter NM, and Landfield PW. (2002) Calcineurin enhances L-type Ca(2+) channel activity in hippocampal neurons: increased effect with age in culture. *Neuroscience* **110,** 213–225.
69. Hell JW, Yokoyama CT, Breeze LJ, Chavkin C, and Catterall WA. (1995) Phosphorylation of presynaptic and postsynaptic calcium channels by cAMP-dependent protein kinase in hippocampal neurons. *EMBO J.* **14,** 3036–3044.
70. Davare MA, Dong F, Rubin CS, and Hell JW. (1999) The A-kinase anchor protein MAP2B and cAMP-dependent protein kinase are associated with class C L-type calcium channels in neurons. *J. Biol. Chem.* **274,** 30,280–30,287.
71. Thibault O, Hadley R, and Landfield PW. (2001) Elevated postsynaptic $[Ca^{2+}]i$ and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity. *J. Neurosci.* **21,** 9744–9756.