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Forum Review

Superoxide Dismutase and Hippocampal Function: Age and Isozyme Matter

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ABSTRACT

Superoxide dismutases (SODs) are the major antioxidant enzymes that inactivate superoxide and thereby control oxidative stress as well as redox signaling. Transgenic mice overexpressing different isozymes of SOD have been used to study the effect of SOD overexpression on hippocampal synaptic plasticity and hippocampus-dependent learning and memory. Studies with transgenic and wild-type animals of different ages show that the function of SOD overexpression changes across the life span of an animal, and comparisons between animals that overexpress different SOD isozymes suggest that the functional value of overexpression as well as the mechanisms through which the respective functional values are effected vary depending on isozyme. The work discussed in this review has important implications for the use of antioxidant treatments and for our understanding of the role of superoxide in physiological and pathological processes. *Antioxid. Signal Redox.* 9, 201–210.

INTRODUCTION

OXIDANTS HAVE BEEN IMPLICATED in a wide variety of pathological processes, including aging and neurodegeneration. Most of these oxidants inevitably form during normal aerobic metabolism and cellular reactions, and some portion is generated by abnormal events, such as exposure to radiation, chemicals, drugs, or anoxic conditions. Common oxidants include superoxide, hydrogen peroxide, and hydroxyl radical. These three species, together with unstable intermediates in the peroxidation of lipids, are referred to as reactive oxygen species (ROS). ROS have been shown to play important roles as both cellular messenger molecules in physiological events, such as activity-dependent synaptic plasticity and memory (17, 21, 54, 62, 73), and toxic molecules in pathological events, such as ischemia, brain injury, and age-related cell damage (7, 14, 18, 20, 23, 28, 67, 78). Because ROS are both beneficial and deleterious to neuronal function, the balance between ROS formation and antioxidant enzymes is critical for normal neuronal function.

Superoxide dismutases (SODs) are a class of oxidoreductases that remove superoxide from organisms through catalyzing the dismutation reaction of the superoxide radical to hydrogen peroxide. The resulting hydrogen peroxide is metabolized to molecular oxygen and water by catalase or glutathione peroxidase (22, 53, 65). SODs are a crucial part of the cellular antioxidant defense mechanism (58). In mammals, there are three different SOD genes encoding three different SOD enzymes known as SOD isozymes. They catalyze the same chemical reaction but display different enzymatic properties and distinct cellular localizations (Fig. 1). Cu/Zn-SOD (SOD-1) is found mainly in intracellular compartments; Mn-SOD (SOD-2) is localized primarily in the mitochondrial matrix; and extracellular SOD (EC-SOD) first was detected in the extracellular fluid. Its expression pattern is highly restricted to specific cell types and tissues (53, 61, 63). In brain, EC-SOD also is found on the endothelial cell surface and in intracellular compartments (62). Cu/Zn SOD and EC-SOD both have copper and zinc in the catalytic center for their activities and are homologous to each other. Mn-SOD

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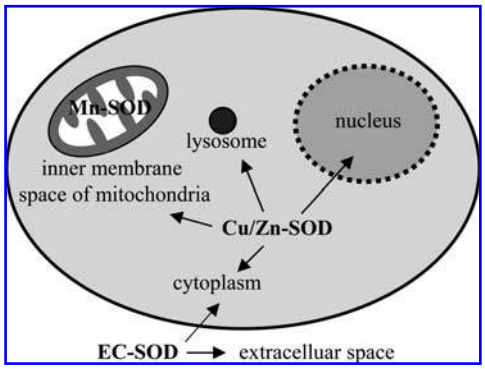

FIG. 1. Cellular localization of the three SOD isozymes. Cu/Zn-SOD is found in all intracellular compartments: the cytoplasm, the nucleus, lysosomes, and the inner membrane space of mitochondria. Mn-SOD is localized primarily in the mitochondrial matrix. EC-SOD is detected mainly in the extracellular space, although in brain, it also is found on the endothelial cell surface as well as in intracellular compartments.

has manganese at the active site, and its amino acid sequence is quite different from those of the other two isozymes.

SODs are believed to be important to protect cells against oxidative damage. It has been shown that an upregulation of SOD may serve to attenuate neuropathological conditions (14, 39, 49, 55, 64), whereas the loss of SOD activity may facilitate neurodegeneration and any associated deterioration of cognitive function due to exacerbation of oxidative damage (19, 43, 69).

In this review, we will focus on superoxide and its regulation by SODs in the context of synaptic and cognitive plasticity. The evidence we will present showcases the dual roles of superoxide and how, across an animal's lifetime, the benefit of superoxide signaling may be outweighed by the accumulation of cellular damage due to superoxide-induced oxidative stress. Not surprisingly, the functional value of SOD upregulation also varies across an animal's lifetime. Age, however, is not the only variable that affects the outcome of SOD upregulation. As we will discuss below, the particular SOD isozyme also impacts on the functional value of SOD upregulation. The issues we raise below have important implications for therapeutic approaches built on manipulation of SODs.

SOD AND HIPPOCAMPAL SYNAPTIC PLASTICITY

The hippocampus is a critical area for certain types of memory function, including consolidation of declarative memory (75); therefore, synaptic plasticity in this brain region is under intense investigation. Hippocampal long-term potentiation (LTP) is a long-lasting increase in synaptic strength that has been proposed as a cellular substrate of learning and memory (9, 51). Several lines of evidence suggest that superoxide contributes to the formation of LTP. First, superoxide was observed in hippocampal slices after *N*-methyl-D-aspartate (NMDA) receptor activation, which is a critical event for the induction of hippocampal LTP (8). Second, our laboratory and others have found that superoxide is able to regulate activation of extracellular signal-regulated kinase (ERK) (38) and autonomous activity of protein kinase C (PKC) (42), both of which are essential for the maintenance and expression of LTP (68). Third, we have shown that adding cell-permeable and cell-impermeable scavengers of superoxide can block/attenuate LTP (40). On the other hand, application of superoxide to hippocampal slices can cause a PKC-dependent LTP-like potentiation (42). Figure 2 summarizes our current understanding of the mechanisms through which superoxide contributes to the formation of LTP. As the major antioxidant enzyme to remove superoxide, SODs are proposed to interfere with hippocampal synaptic plasticity.

In the next two sections, we will discuss the role of SODs in hippocampal synaptic plasticity and the different effects of SOD upregulation on hippocampal synaptic plasticity as a function of age and SOD isozyme.

SOD and hippocampal synaptic plasticity in young animals

Transgenic mouse models overexpressing SODs provide a good tool for studying the relationship between SOD and hippocampal function. We studied LTP in young adult (2- to 4-month-old) mice that overexpressed EC-SOD (33, 77). EC-SOD activity in the hippocampus of heterozygous EC-SOD transgenic mice is about 10-fold higher than that in the hippocampus of wild-type mice (77). We examined LTP in hippocampal slices from these mice using high-frequency stimulation (HFS) delivered to the Schäffer collateral/commissural fibers to induce LTP in hippocampal area CA1. HFS-induced LTP is the most extensively studied form of LTP. HFS typically consists of one train or several trains of a 100-Hz tetanus, which causes activation of the NMDA receptor and a consequent transient rise in postsynaptic calcium. The rise in calcium, in turn, triggers the production of other small mes-

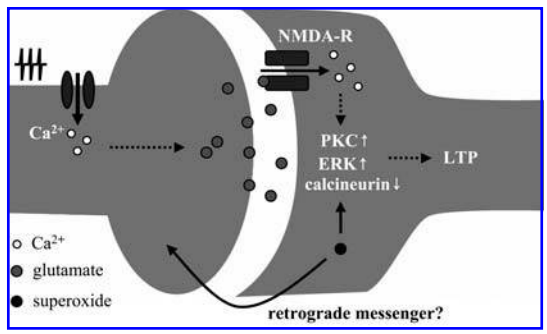

FIG. 2. Hypothesized superoxide function in LTP. Production of superoxide by possible sources such as NADPH oxidase, mitochondria, xanthine/xanthine oxidase, nitric oxide synthase, and arachidonic acid metabolism (not shown in this graph) enhances the phosphorylation of downstream enzymes critical for LTP by either activating protein kinases or inactivating protein phosphatases. Superoxide may also function as a retrograde messenger to regulate neurotransmitter release in presynaptic neurons. (↑), superoxide causes an increase, and (↓), superoxide causes a decrease in the activity of the indicated enzyme. (?), it has not yet been determined whether superoxide participates in the indicated pathway.

senger molecules, such as cyclic adenosine monophosphate (cAMP), nitric oxide, arachidonic acid, and redox molecules (68). These small messenger molecules, as well as calcium itself, have been shown or proposed to activate protein kinases associated with or necessary for the expression of LTP (68). Using HFS to alter synaptic strength, we found that LTP was impaired greatly in young adult EC-SOD transgenic mice, whereas it developed normally in their wild-type littermates (77).

To exclude the possibility that the deficiency we saw was due to abnormal baseline synaptic transmission or presynaptic plasticity, such as altered post-tetanic potentiation (PTP) or paired-pulse facilitation (PPF), we also compared between the two genotypes the magnitude of synaptic currents mediated by AMPA and NMDA receptors, the amount of total and steady-state depolarization during HFS, the magnitude of PTP, and the magnitude of PPF. We found no evidence that either altered basal transmission, current flow during HFS, or presynaptic plasticity was responsible for impaired LTP in EC-SOD transgenic mice (77).

Hydrogen peroxide has been shown to interfere with LTP in area CA1 (36). Therefore, another possible explanation for deficient LTP in EC-SOD transgenic mice was that the levels of hydrogen peroxide, one of the metabolic products of superoxide dismutation reaction, might be increased in these animals (2). If that were the case, then removing hydrogen peroxide with catalase would restore normal LTP in EC-SOD transgenic mice. However, experiments in which we bathed brain slices from EC-SOD transgenic mice in catalase-containing buffer did not provide support for this explanation, because LTP still was impaired (77).

To test the possibility that the blockade of LTP could be attributed to enhanced GABAergic neurotransmission in transgenic animals, we delivered LTP-inducing HFS to slices from wild-type and EC-SOD transgenic mice in the presence of bicuculline, a selective $GABA_A$ receptor antagonist. Bicuculline had no effect on the blockade of LTP in slices from EC-SOD transgenic mice, which suggests that the LTP deficits in area CA1 of transgenic mice were not due to a change in GABAergic inhibition in these animals (77).

Finally, to rule out the possibility that EC-SOD overexpression interfered with the machinery necessary for synaptic plasticity by mechanisms unrelated to superoxide metabolism, we incubated hippocampal slices in solution that contained the copper chelator diethylcarbamate (DDCA), which inhibited EC-SOD activity by removing copper ions. In the presence of DDCA, HFS induced LTP in slices from EC-SOD transgenic mice that was indistinguishable from the level of LTP in hippocampal slices from wild-type mice (77). Taken together, these findings show that EC-SOD overexpression leads to impairment in hippocampal synaptic plasticity, and it does so because it accelerates superoxide dismutation.

Similar to the deleterious effect of EC-SOD overexpression on hippocampal LTP, overexpression of Cu/Zn-SOD was found to be associated with impaired LTP in hippocampal area CA1 in 2-month-old transgenic mice (37). However, in the case of Cu/Zn-SOD overexpression, the LTP deficit could be rescued by bathing the hippocampal slices of the transgenic animals in either catalase, an ROS-spin-trapping agent, or inhibitors of $GABA_A$ receptors (25, 48). These findings suggest that Cu/Zn-SOD upregulation may cause an increase in the formation of hydrogen peroxide, as has been reported previously (24), and the increase in hydrogen peroxide concentration, in turn, interferes with hippocampal LTP. This idea was confirmed by experiments in which application of high concentrations of hydrogen peroxide were found to interfere with the induction of LTP in hippocampal slices from wild-type animals (36).

The foregoing observations imply mechanistically different effects of the genetic manipulation of different SOD isozymes. In EC-SOD transgenic mice, too little superoxide results in impaired LTP, whereas in Cu/Zn-SOD transgenic mice, too much hydrogen peroxide leads to diminished LTP. It is possible that such a difference in mechanism is caused by a difference in the amount and/or location of peroxide formation or degradation by the two SOD isozymes. Despite the apparent difference in the mechanism of action between the two SODs, the principal conclusion emerging from these studies with SOD transgenic mice is that normal ROS signaling is critical for LTP in hippocampal area CA1. Indiscriminate suppression of superoxide by overexpression of SOD therefore may not always be an appropriate therapeutic method for preventing oxidative stress.

Superoxide in the extracellular space and in the cytosol, regulated by EC-SOD and Cu/Zn-SOD, are not the only pools of superoxide; mitochondrial superoxide constitutes another pool of superoxide that is generated during normal aerobic metabolism. Mitochondria have been proposed to be an important link between oxidants and aging (3). It is estimated that the majority of intracellular ROS production is derived from mitochondria, where the generation of ATP by oxidative phosphorylation takes place (82). As a by-product, a small fraction of oxygen consumption is converted to ROS in and around this organelle (15). Under physiological conditions, this fraction has been estimated to be around 0.2% (72, 74), although under conditions of altered cellular metabolism, mitochondrial generation of ROS may be considerably higher (1). As a consequence, mitochondria are enriched fully with antioxidants, including Mn-SOD, Cu/Zn-SOD, catalase, peroxiredoxin, glutathione, and glutathione peroxidase, to minimize oxidative stress to the cell (82). There is a debate whether mitochondrial superoxide is involved in HFS-induced LTP, since the phospholipid bilayer structure of the mitochondrial membranes does not allow free diffusion of superoxide generated within mitochondria (82). To help resolve this debate, we recently have begun to examine the role of mitochondrial superoxide in hippocampal synaptic plasticity using transgenic mice that overexpress mitochondrial SOD, also referred to as Mn-SOD.

Immunogold electron microscopy confirmed that the transgenic mice overexpress Mn-SOD restricted to mitochondria (52, 59). Enzyme activity assays revealed that hemizygous Mn-SOD transgenic mice display a 2.3-fold increase in Mn-SOD activity in total brain homogenates (52). When comparing baseline synaptic transmission and two forms of presynaptic plasticity (PPF and PTP) in area CA1 between hippocampal slices from 3-month-old mice with Mn-SOD overexpression versus their wild-type littermates, we observed no significant effect of genotype (Hu *et al*., unpub-

lished observations). These findings are similar to what we found in EC-SOD overexpressing mice and suggest that overexpression of Mn-SOD does not exert an adverse effect on basic synaptic function and presynaptic plasticity in area CA1. However, different from our observations with EC-SOD overexpressing mice, we found no effect of Mn-SOD overexpression on LTP induced by HFS of the Schaffer collateral/commissural pathway. The induction and expression of LTP was indistinguishable between the two genotypes (Hu *et al*. unpublished observations).

In summary, the three SOD isozymes have distinct signaling functions in hippocampal LTP, as upregulation of each of them results in different phenotypes. In the young adult hippocampus, upregulation of Cu/Zn-SOD or EC-SOD impairs LTP because of accumulation of excessive hydrogen peroxide or reduction of superoxide, respectively. Upregulation of Mn-SOD, on the other hand, has no obvious effect on hippocampal LTP.

In addition to playing a direct role in the signaling events that underlie hippocampal LTP in young adult animals, SODs affect hippocampal plasticity indirectly in aged animals. In the next section, we will discuss the antioxidative role of SODs and its impact on synaptic plasticity in aged animals.

SOD and hippocampal synaptic plasticity in aged animals

Impaired LTP is one of the typical hippocampal deficits observed in aged animals (4). Examination of levels of ROS and oxidative stress markers in young versus aged brain has revealed that both of these measures are increased in aged animals (70). Age-related LTP impairments in hippocampal area CA1 and the dentate gyrus have been attributed in part to an age-dependent increase in ROS levels (2, 37). If this explanation is correct, then it is conceivable that antioxidant treatment may help protect against age-related LTP impairment.

We tested this protection hypothesis by comparing LTP in area CA1 of 2-year-old EC-SOD overexpressing mice to that of their age-matched wild-type littermates. We found that aged EC-SOD transgenic mice exhibit enhanced LTP during both the early phase of LTP (E-LTP) and the late phase of LTP (L-LTP) compared to the level of LTP in their wild-type littermates (33). Similar to young EC-SOD transgenic mice, we did not observe changes in either basal synaptic transmission or presynaptic plasticity in aged transgenic mice compared to their wild-type age-matched littermates. Furthermore, we ruled out the possibility that the enhanced LTP was caused by altered hydrogen peroxide metabolism by testing the effect of HFS on synaptic transmission in hippocampal slices in the presence of catalase. We found catalase to have no effect on LTP (33). We also examined constitutive superoxide production and brain protein oxidation across age. The biochemical measures revealed that aging in wild-type mice is associated with a dramatic increase in constitutive superoxide formation, one that was blunted markedly by EC-SOD overexpression (33). Aging in wild-type mice also was associated with an increase in protein oxidation in whole brain homogenates, and this effect was abolished completely by EC-SOD overexpression (33). Taken together, these findings suggest that life-long EC-SOD overexpression can have beneficial effects on hippocampal synaptic plasticity in old age, and that these beneficial effects may be due to a reduction in superoxide-mediated protein oxidation and consequent neuronal damage in the hippocampus.

The results with young EC-SOD transgenic mice showed that the SOD overexpression leads to LTP deficits, whereas the findings with aged EC-SOD transgenic mice showed that the SOD overexpression leads to improved LTP compared to the level of LTP in age-matched wild-type animals. The seemingly contradictory phenotypes of young EC-SOD transgenic mice versus aged EC-SOD transgenic mice reveal an age-dependent alteration in the function of superoxide and SOD. In young EC-SOD transgenic mice, SOD overexpression reduced the physiological level of superoxide, a signaling molecule critically involved in synaptic plasticity. As a consequence, EC-SOD overexpression impaired LTP. However, in aged EC-SOD mice, basal level of superoxide was found to be elevated relative to those in young EC-SOD mice (33), and this elevation appears to be sufficient to allow normal expression of LTP despite the excess of the superoxide-scavenging enzyme (33). On the other hand, because total superoxide level is reduced in aged EC-SOD transgenic mice compared with aged wild-type mice, there was a partial protection against oxidative damage (33). The more robust LTP in aged transgenic animals may therefore be a result of the protection against neuronal oxidative stress in the presence of excess EC-SOD.

Interestingly, similar changes in hippocampal LTP induced by theta burst stimulation (TBS) have been reported in aged transgenic mice overexpressing Cu/Zn-SOD (37). TBS is composed of several bursts of four pulses at 100 Hz, with the bursts delivered at 5 Hz. Like HFS-induced LTP, LTP induced by TBS requires activation of the NMDA receptor. TBS, however, is believed to reflect more closely physiological processes during learning and memory than does HFS. Two-year-old Cu/Zn-SOD mice were shown to exhibit enhanced TBS-LTP compared to the level of LTP that developed in their wild-type littermates. However, when the action of hydrogen peroxide on LTP was explored, paradoxical results emerged. As mentioned earlier, in young wild-type animals the exogenous application of hydrogen peroxide to hippocampal slices was found to inhibit LTP (36, 79). However, in aged wild-type animals, extra hydrogen peroxide was found to cause no effect on HFS-induced LTP (79) and to reverse the age-dependent impairment of LTP induced by TBS (37). When the same manipulations were applied to Cu/Zn-SOD transgenic animals, it was found that in young transgenic animals, extra hydrogen peroxide restores the LTP impairment normally seen in young Cu/Zn-SOD transgenic mice, whereas in aged transgenic animals, extra hydrogen peroxide markedly attenuated LTP (37). These findings suggest a critical regulatory role in LTP by hydrogen peroxide, one that changes across an animal's lifetime as well as under conditions of Cu/Zn-SOD overexpression, possibly due to constitutively increased hydrogen peroxide production with overexpression of this SOD isozyme (24). However, additional studies are needed to shed light on the complex relation between hippocampal synaptic plasticity, superoxide, Cu/Zn-SOD, hydrogen peroxide, and age.

The effect of overexpression of Mn-SOD on synaptic plasticity in aged animals also has begun to be examined. Using

the same LTP induction protocol as used with EC-SOD transgenic mice, we found no effect of Mn-SOD overexpression on LTP (Hu *et al*., unpublished observations). Two-year-old Mn-SOD transgenic mice exhibited a similar age-dependent decline in LTP as did wild-type controls, which suggests that overexpression of Mn-SOD, in apparent contrast to overexpression of EC-SOD or Cu/Zn-SOD, might not protect against tissue deterioration in the hippocampus of aged animals.

It is interesting to note, however, that when subjected to a number of different treatments that produce either acute toxicity or oxidative stress, Mn-SOD transgenic mice showed enhanced resistance and decreased tissue damage in virtually all cases (12, 16, 32, 52, 81). For instance, it has been reported that using the same transgenic line as used in our experiments, Mn-SOD overexpression protects against 6-hydroxydopamine-induced brain injury (12) and methamphetamine-induced brain damage (52). These results suggest that Mn-SOD overexpression can protect against acute toxicity and stressors in certain brain regions, but Mn-SOD overexpression might not impact on the physiological process of aging.

Taken together, the evidence thus far suggests that the functional value of SOD in synaptic plasticity depends on the age of the animal and the type of SOD. The nature and/or brain region of damage may reveal to be a relevant factor as well. In the next section, we will discuss how the effect of SOD overexpression is manifested at the behavioral level.

SOD AND MEMORY

The role of superoxide has also been explored at the behavioral level, in particular with respect to limbic system-dependent cognitive function. Similar to the theme that has emerged from the study of superoxide function at the neuronal circuit level, the behavioral work suggests that superoxide plays two very different roles: it is critical for normal memory function, but it also can contribute to neuronal damage, thereby disrupting the very network in which it plays a signaling role for normal function. These insights are based on studies in which the behavioral consequences of genetic or pharmacologic manipulations of SOD have been examined. Studies in which specific SOD isozymes have been targeted furthermore support the second theme that has emerged from the synaptic studies, namely, that the functional consequence of SOD overexpression varies depending on the SOD isozyme. In the next two sections, we summarize behavioral work from our own lab and that of others in support of these two themes.

SOD and memory in young animals

In the mid 1990s, a series of pharmacological studies suggested a correlation between SOD and memory function. Research on the effect of pesticides found that increased SOD levels co-varied with impaired memory function in young rats (27); investigations of the effect of Jianyi oral liquid, an agent used in traditional Chinese medicine, indicated that increased SOD levels co-varied with improved memory in aged mice and humans (11); and work on the Chinese herbal extract anisodamine found that this agent blunts memory deficits after experimentally-induced cerebral ischemia in rats, presumably by enhancing SOD activity and thereby preventing superoxide-mediated neurotoxicity (83).

Although this early work was suggestive, it was not until the development of SOD transgenic mice in the late 1990s that a clear relationship between SOD and, by extension, superoxide, and memory function was established. Focusing on Cu/Zn-SOD, which is elevated in the brain of individuals with Down syndrome and for which the encoding gene is located on chromosome 21 (10, 34), Gahtan *et al*. (25) demonstrated that young adult (about 4-month-old) mice that overexpress this SOD isozyme exhibit impaired performance in a water maze task designed to test the acquisition of spatial memory, a hippocampus-dependent form of memory. As discussed above, LTP in area CA1 of hippocampal slices prepared from these mice was also found to be impaired (25), which suggests a link between these two functional consequences of Cu/Zn-SOD overexpression. The synaptic deficit could be overcome by treatment of the hippocampal slices with either an antioxidant spin-trapping agent or catalase (25), which raises the interesting question whether normal hippocampus-dependent learning can also be restored in Cu/Zn-SOD overexpressing mice through pretreatment with these agents. We are unaware of studies that have addressed this possibility, despite the important implications they might have for the treatment of the cognitive deficits observed in Down syndrome.

Similar to the deleterious effect of Cu/Zn-SOD overexpression on hippocampus-dependent memory in young adult animals, this type of cognitive function was found to be impacted negatively by EC-SOD overexpression in young adult mice. Levin *et al*. (44, 45) described dramatic deficits in the acquisition of spatial memory needed for correct performance in a radial-arm maze task in young adult (about 2- to 4-month-old) EC-SOD transgenic mice. Using as cognitive assay contextual fear conditioning, which, too, depends on the integrity of the hippocampus, we found that EC-SOD overexpression greatly interferes with the consolidation of contextual fear conditioning in 2- to 4-month-old mice (76, 77). The impairment in fear memory consolidation could not be attributed to reduced pain sensitivity, altered basal activity level or exploratory behavior, or impaired short-term contextual memory (77). As described above, we also noted that LTP in area CA1 is impaired in hippocampal slices from young EC-SOD transgenic mice (77). Taken together, these findings are consistent with the idea that EC-SOD overexpression leads to impairment in memory function because of its interference with hippocampal synaptic plasticity.

This idea is bolstered by our observations that SOD activity is markedly increased and constitutive superoxide formation decreased in 2- to 4-month-old EC-SOD transgenic mice (77), that pharmacological inhibition of superoxide interferes with hippocampal LTP (40), and the finding that NMDA receptor activation, a critical element in the chain of events that lead to LTP and the establishment hippocampus-dependent memory, causes superoxide production (8, 42). Interestingly, the LTP impairment could be overcome by treatment of hippocampal slices from EC-SOD transgenic mice with the copper chelator diethylcarbamate (77). This observation raises

the yet untested question whether normal memory function can likewise be rescued by pretreatment of the transgenic animals with agents that reduce EC-SOD activity, such as chelators of EC-SOD cofactors.

Different from the pronounced effects of overexpression of either Cu/Zn-SOD or EC-SOD on hippocampus-dependent memory, we found no evidence for altered memory function in 3-month-old mice that overexpress Mn-SOD (Hu *et al*., unpublished observations). Contextual fear conditioning developed normally and was indistinguishable from that of wild-type littermates when tested 24 h after training. Thus, extraordinary scavenging of mitochondrial superoxide by Mn-SOD does not appear to yield a phenotype at either the synaptic level (described above) or the cognitive level, at least not early in adulthood.

SOD and memory in aged animals

Whereas high levels of EC-SOD and consequent enhancement of superoxide scavenging are detrimental to normal memory function in young and uninjured animals, they appear to be protective of normal memory function in aged or injured animals. The incidence of cognitive impairment, in particular of impairment in hippocampus-dependent memory function, is well known to be increased in old age and is associated with a number of neurodegenerative disorders and conditions, such as Alzheimer's disease, ischemia, and traumatic brain injury. Highly relevant to the present discussion, ROS, including superoxide, have been implicated in the cell damage observed in each of these conditions (7, 14, 28, 30, 50, 57, 78). Not surprisingly, then, treatment with antioxidants is considered widely to be one of the most promising therapeutic strategies for these conditions.

SOD overexpressing mice provide an excellent tool for testing the potential effectiveness of such a strategy, including its ability to prevent the cognitive deficits associated with these conditions. Consistent with the idea that superoxide scavengers preserve or enable normal function under conditions of compromised function due to an overabundance of ROS activity, Levin *et al*. (46, 47) reported that aged (>24-month-old) EC-SOD transgenic mice outperform aged wild-type mice on the radial-arm maze task in that they commit fewer errors over the course of task acquisition as well as at asymptotic level. Whereas aged wild-type mice were found to exhibit a marked age-dependent decline in performance of this spatial memory task, aged EC-SOD transgenic mice showed no decline in performance from 12 months of age to 30 months of age (46).

Using the spatial water maze paradigm, we found that EC-SOD overexpression has no effect on the apparent rate of acquisition of the spatial memory, but that it does attenuate the age-dependent decline in the strength or accuracy of the memory observed in wild-type animals (33). Specifically, wild-type mice exhibited a decrease from 13–14 months of age to 19–20 months of age in the amount of time they spent in the quadrant of the maze in which the escape platform previously was located. In contrast, EC-SOD transgenic mice showed little change in discriminative performance across age; as a result, the amount of time that aged EC-SOD transgenic mice spent in the target quadrant was greater than that of their age-matched, wild-type littermates (33). We also assessed consolidation of contextual fear conditioning in 2-year-old mice but, different from the spatial memory paradigms, did not observe an age-dependent decline in memory in wild-type mice. Furthermore, aged EC-SOD transgenic mice continued to display impaired consolidation of the fear memory, although the magnitude of the deficit was attenuated compared to the deficit seen in young EC-SOD transgenic mice (33). Work by others has shown that an age-dependent deficit in the retention of contextual memory may not emerge unless the task involves intervals between training and testing that are longer than the 24-h interval we used (26, 60), which could explain why we did not see an age-effect in our study. In light of this possibility, it would be of interest to determine whether at retention intervals long enough to reveal a deficit in normal aged animals, this deficit is attenuated in aged EC-SOD transgenic mice.

Interestingly, EC-SOD overexpression appears to protect against not only the loss in cognitive function in old age but also the cognitive impairment induced by traumatic brain injury. Comparing the effect of a moderate traumatic impact in the forebrain on the acquisition of spatial memory in the water maze task, Pineda *et al*. (66) found that EC-SOD transgenic mice exhibit slightly accelerated acquisition of this task compared to their wild-type littermates subjected to the same kind of closed head injury. Whether a similar benefit from EC-SOD overexpression is seen with respect to the cognitive deficits that ensue after an ischemic insult in the forebrain remains to be tested.

The effect of overexpression of Cu/Zn-SOD on cognitive function in aged mice has not yet been assessed. A recent analysis of hippocampal proteins in Cu/Zn-SOD overexpressing mice, however, suggests that overexpression of this SOD isozyme can cause decreases in a number of key neuronal proteins, including synaptosomal proteins, possibly due to increased hydrogen peroxide production by Cu/Zn-SOD (24, 71). Any benefit from enhanced superoxide scavenging by SOD may thus be outweighed by the increase in the production of this non-radical ROS, which itself has neurotoxic effects (29). Indeed, elevated hydrogen peroxide formation has been implicated in both Alzheimer's disease and Down syndrome (5, 13, 56). The possibility that overexpression of Cu/Zn-SOD, in contrast to overexpression of EC-SOD, may not protect against age-related loss of function also is suggested by findings that combined overexpression of Cu/Zn-SOD and amyloid precursor protein (APP) did not alleviate any impairment in either hippocampus-dependent memory or hippocampal LTP observed in single APP-overexpressing mice (31). Instead, the impairments in the double-mutants either matched or surpassed those observed in the single, Cu/Zn-SOD- or APP-overexpressing mutants (31). Similarly, no benefit was observed from Cu/Zn-SOD overexpression on the loss in recognition memory after closed head injury. The ability to distinguish a novel from a familiar object was equally disrupted immediately after the traumatic impact in the forebrain of wild-type and Cu/Zn-SOD transgenic mice, and the recovery to normal, pre-injury levels of memory function appeared to proceed at a similar rate in both genotypes (6). Interestingly, the degree of neuropathology after the closed head injury was found to be attenuated markedly in

Cu/Zn-SOD *deficient* mice, a finding that is consistent with the idea that the neuropathology is mediated, at least in part, by hydrogen peroxide whose production is promoted by Cu/Zn-SOD (6, 24). Thus, although both EC-SOD and Cu/Zn-SOD scavenge superoxide, the behavioral consequences of their overexpression appear to differ, especially under conditions of oxidative stress. As discussed above, this kind of divergence in outcome was not observed at the physiological level. The age-dependent decline in LTP in area CA1 was found to be abolished in aged Cu/Zn-SOD transgenic mice (37), similar to what we found in aged EC-SOD transgenic mice (33).

Whereas EC-SOD overexpression appears to mostly protect against age- or injury-related cognitive deficits and Cu/Zn-SOD overexpression may possibly exacerbate them, Mn-SOD overexpression appears to have no effect on memory function, whether in young or aged animals. Similar to the lack of a phenotype in young adults (see above), we found no difference in either contextual fear conditioning or performance in the spatial water maze task between 2-year-old Mn-SOD transgenic mice and their age-matched wild-type littermates (Hu *et al*., unpublished observations). These findings invite the conclusion that superoxide produced in the mitochondrial compartment is neither essential in these physiologic functions nor does any increase in mitochondrial superoxide that might develop across age contribute critically to age-related neurodegeneration. In the next section, we will focus on the main insights that can be derived from the collective findings summarized above.

SUMMARY AND CONCLUSIONS

There is growing recognition that ROS function as signaling molecules not only in apoptosis pathways but also in normal physiological processes (35, 41, 42, 70). The foregoing discussion has provided ample examples that show superoxide to be critical for normal hippocampal synaptic plasticity and normal hippocampus-dependent memory. These examples suggest a positive relation between level of superoxide and strength of LTP or memory. At the same time, it has been documented extensively that high levels of ROS, including of superoxide, are toxic and cause cell death (28). This literature suggests a positive relation between level of superoxide and degree of pathology. How are these two roles of superoxide to be reconciled?

The answer may lie, at least in part, in the magnitude and the duration of the superoxide signal. The nature of the superoxide signal involved in physiological functions is likely to be brief and relatively small. These brief small signals, in isolation, are unlikely to produce damage. On the other hand, repeated, long-lasting, and/or large elevations of superoxide lead to oxidative stress and, eventually, apoptosis, thereby destroying the cellular network within which superoxide serves as a physiological messenger (Fig. 3A).

In an effort to harness the latter function of ROS, the effect of increased availability of antioxidants has been examined in clinical as well as experimental settings, with the finding that, generally, high levels of ROS scavengers protect against the damaging effects of ROS. Findings from our lab and oth-

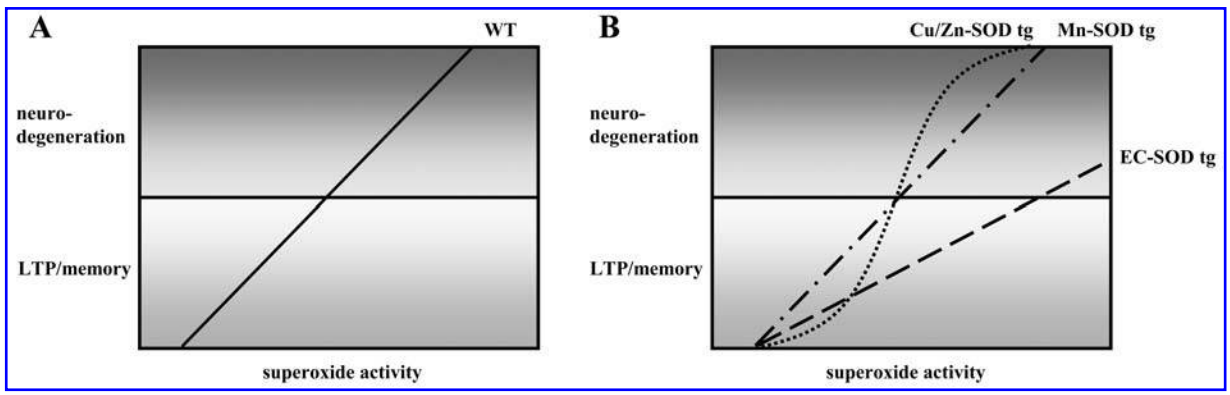

FIG. 3. Schematic representation of relation between level of superoxide activity and level or strength of LTP and memory (*light gray*) and neurodegeneration (*dark gray*). (**A**) Depiction of the relation between superoxide activity and the functional outputs considered in this review in wild-type animals. When superoxide activity generally is low, as is the case early in life, superoxide serves as a critical signaling molecule in physiological processes, such as synaptic and cognitive plasticity. When superoxide activity generally is high, as is the case late in life, superoxide plays a critical signaling role in neurotoxic processes. (**B**) Depiction of the relation between superoxide activity and the respective functional outputs under conditions of overexpression of either of three SOD isozymes. Whereas overexpression of the superoxide scavenger EC-SOD interferes with LTP and memory when superoxide activity generally is low (*i.e.*, early in life), it protects against neurodegeneration and associated loss of synaptic and cognitive plasticity when superoxide levels are high (*i.e.*, late in life) (*stippled line*). Overexpression of Mn-SOD appears to have little impact, relative to what is observed in wild-type animals, on the relation between superoxide activity and the functional outputs discussed in this review (*stipple-dotted line*). Overexpression of Cu/Zn-SOD causes disruption of LTP and memory early in life, when superoxide activity generally is low (*dotted line*), similar to the effect of overexpression of EC-SOD. However, overexpression of Cu/Zn-SOD late in life, when superoxide activity generally is high, does not prevent neurodegeneration but instead may promote it. The failure of a protective effect of Cu/Zn-SOD overexpression may be related to the overproduction of hydrogen peroxide, which has been reported in association with Cu/Zn-SOD overexpression. See text for further details.

ers showing that synaptic and memory function are preserved above normal levels in *aged* EC-SOD overexpressing mice are consistent with this principle. The biochemical data suggest that chronic EC-SOD overexpression prevents the damage that normally ensues upon life-long exposure to superoxide (33). Under those conditions, the infrastructure necessary for LTP and memory appears to be preserved across age, and hence these physiological events can proceed normally.

In the absence of chronically or profoundly elevated levels of ROS, an increase in ROS scavengers has no protective function but counteracts any physiological signals that ROS might have. Again, the findings from our laboratory and others showing that EC-SOD overexpression interferes with LTP and memory in *young* animals is consistent with this principle. Thus, one might envision a relation between superoxide levels, EC-SOD overexpression, and functional variables, such as hippocampal LTP and memory, as illustrated in Fig. 3B (stippled line). EC-SOD overexpression essentially ensures that even at constitutively high superoxide levels, as apply in old age, superoxide does not cause damage but remains in the realm of functioning as a signaling molecule in physiological events.

The findings we have discussed in this review, summarized in Table 1, suggest that the relation depicted in Fig. 3B (stippled line) does not apply to all isozymes of SOD. The differential outcome in ROS scavenging by EC-SOD versus Cu/Zn-SOD versus Mn-SOD may be attributable, at least in part, to the difference in subcellular compartments in which these isozymes scavenge superoxide and in part to an apparent difference in hydrogen peroxide-generating tendency between the isozymes. Whereas Mn-SOD scavenges mitochondrial superoxide, EC-SOD in brain and Cu/Zn-SOD scavenge cytosolic superoxide (62). Mitochondrial superoxide does not appear to play a role in the physiological variables we have discussed in this review. Accordingly, overexpression of the SOD that targets this superoxide has no detectable consequences on these functional variables (Fig. 3B, stipple-dotted line; but see 12, 16, 32, 52, 81).

The consequences of overexpression of Cu/Zn-SOD appear to be more complex, possibly because at low concentrations, Cu/Zn-SOD appears to primarily scavenge superoxide whereas at higher concentrations, it appears to lead to hydrogen peroxide formation and associated neuronal damage (24, 25, 31, 71, 80; but see 37). Thus, to interpret the effects of Cu/Zn-SOD overexpression, knowledge of the additional variable of hydrogen peroxide production is critical. Therefore, whether the effect of Cu/Zn-SOD overexpression on the relation between superoxide and the hippocampal functions we discussed here is best represented by a simple left-shift of the relational line in Fig. 3A or by a transformation of the straight line into a sigmoidal line (Fig. 3B, dotted line) is currently unclear. Future studies directed at elucidating the consequences of SOD overexpression on both superoxide production and hydrogen peroxide production evoked by acute events, such as LTP-inducing stimulation or learning, as well as across prolonged states, such as young vs. old adulthood, should help resolve this apparent paradox.

In conclusion, the use of SOD transgenic animals has led to important insights into the role of superoxide in physiological and pathological processes. Better understanding of the various biochemical effects of SOD overexpression, in addition to its effect on superoxide activity, should advance further our understanding of the mechanisms that underlie the diverse functional consequences of SOD overexpression.

Table 1. The Effect of SOD Overexpression on LTP and Memory in Young and Old Mice

SOD isozyme	*Young*		*Old*	
	LTP	*Memory*	*LTP*	*Memory*
Cu/Zn-SOD	↓	↓	↑	N.D.
Mn-SOD	−	−	−	−
EC-SOD	↓	↓	↑	↑

This table summarizes the effects of overexpression of different SOD isozymes on hippocampal LTP and hippocampus-dependent memory in young adult and old adult mice. (↑), the respective SOD overexpression had a beneficial effect relative to the functional level observed in age-matched wild-type animals; (↓), the respective SOD overexpression had a detrimental effect; (−), the respective SOD overexpression had no detectable effect on the particular function; (N.D.), not determined.

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ABBREVIATIONS

APP, amyloid precursor protein; Cu/Zn SOD, copper- and zinc-containing superoxide dismutase; DDCA, copper chelator diethylcarbamate; EC-SOD, extracellular superoxide dismutase; ERK, extracellular signal-regulated kinase; GABA, gamma-aminobutyric acid; HFS, high-frequency stimulation; LTP, long-term potentiation; Mn-SOD, manganese-containing superoxide dismutase; NMDA, *N*-methyl-D-aspartate; PKC, protein kinase C; PPF, paired-pulse facilitation; PTP, post-tetanic potentiation; ROS, reactive oxygen species; SOD, superoxide dismutase; TBS, theta burst stimulation.

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