

Hippocampal long-term potentiation, memory, and longevity in mice that overexpress mitochondrial superoxide dismutase

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Abstract

Superoxide has been shown to be critically involved in several pathological manifestations of aging animals. In contrast, superoxide also can act as a signaling molecule to modulate signal transduction cascades required for hippocampal synaptic plasticity. Mitochondrial superoxide dismutase (SOD-2 or Mn-SOD) is a key antioxidant enzyme that scavenges superoxide. Thus, SOD-2 may not only prevent aging-related oxidative stress, but may also regulate redox signaling in young animals. We used transgenic mice overexpressing SOD-2 to study the role of mitochondrial superoxide in aging, synaptic plasticity, and memory-associated behavior. We found that overexpression of SOD-2 had no obvious effect on synaptic plasticity and memory formation in young mice, and could not rescue the age-related impairments in either synaptic plasticity or memory in old mice. However, SOD-2 overexpression did decrease mitochondrial superoxide in hippocampal neurons, and extended the lifespan of the mice. These findings increase our knowledge of the role of mitochondrial superoxide in physiological and pathological processes in the brain.

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1. Introduction

Reactive oxygen species (ROS) include superoxide, hydrogen peroxide, hydroxyl radicals, and unstable intermediates derived from the peroxidation of lipids. ROS have been shown to play important roles as both cellular messenger molecules in physiological events, such as activity-dependent synaptic plasticity and memory (Droge, 2002; Forman, Torres, & Fukuto, 2002; Martindale & Holbrook, 2002; Oury, Card, & Klann, 1999; Stone &

Yang, 2006), and toxic molecules in pathological events, such as ischemia, brain injury, and age-related cell damage (Esposito, Ammendola, Faraonio, Russo, & Cimino, 2004; Finkel & Holbrook, 2000; Fukagawa, 1999; Pratico, 2002). Thus, ROS can be both beneficial and deleterious to neuronal function, with the balance between ROS and antioxidants critical for maintaining normal neuronal function.

Superoxide dismutases (SODs) are a class of oxidoreductases that remove superoxide from organisms by catalyzing the dismutation of the superoxide radical to hydrogen peroxide. The resulting hydrogen peroxide is metabolized to molecular oxygen and water by either catalase or glutathione peroxidase (Fridovich, 1995; Marklund,

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1984; Petersen et al., 2003). SODs are a crucial part of the cellular antioxidant defense mechanism (Muscoli et al., 2003). In mammals, there are three different SOD genes encoding three different enzymes. These SOD isozymes catalyze the same chemical reaction, but display different enzymatic properties and distinct cellular localizations. SOD-1 (Cu/Zn-SOD) is found mainly in intracellular compartments; SOD-2 (also referred to as Mn-SOD) is localized primarily in the mitochondrial matrix; and extracellular SOD (EC-SOD), which is usually found in the extracellular space, but can also be found in intracellular vesicle-like structures and on cell surfaces (Oury et al., 1999).

Mitochondrial respiration is a major source of intracellular ROS production (Liu, Fiskum, & Schubert, 2002). Under physiological conditions, approximately 0.2% of oxygen consumption is converted to ROS in and around mitochondria (Staniek & Nohl, 2000; St-Pierre, Buckingham, Roebuck, & Brand, 2002). Under conditions of altered cellular metabolism, mitochondrial generation of ROS may even be considerably higher (Albers & Beal, 2000). As a consequence, mitochondria are enriched with antioxidants in order to tightly regulate those free radicals (Zeevalk, Bernard, Song, Gluck, & Ehrhart, 2005). SOD-2 or is the major antioxidant enzyme that controls the release of mitochondrial superoxide (Weisiger & Fridovich, 1973) and therefore, is important for mitochondrial superoxide-regulated physiological and pathological events.

It has been reported that two different lines of SOD-2 homozygous knockout mice die shortly after birth (Li et al., 1995; Lebovitz et al., 1996), whereas overexpression of SOD-2 extends the lifespan of flies (Sun, Folk, Bradley, & Tower, 2002; Sun, Molitor, & Tower, 2004) and attenuates drug-induced neurotoxicity in mice (Callio, Oury, & Chu, 2005; Klivenyi et al., 1998; Maragos et al., 2000). However, questions concerning the neurological effects of chronic SOD-2 overexpression over the lifetime of an animal have yet to be addressed.

Our recent work on another SOD isozyme, extracellular SOD (EC-SOD), revealed that EC-SOD overexpression improves hippocampal synaptic plasticity and memory-related behavioral performance in aged mice (Hu, Serrano, Oury, & Klann, 2006). In light of our observations with EC-SOD transgenic mice, we utilized SOD-2 overexpressing mice to examine the role of mitochondrial superoxide on hippocampal function during aging. We found that SOD-2 overexpression decreased mitochondrial superoxide levels, but had no obvious impact on hippocampal LTP and memory in either young or aged mice. However, similar to the previous findings in flies, SOD-2 overexpression extended the lifespan of mice. We conclude that although mitochondrial superoxide may not contribute to age-related impairments in hippocampal synaptic plasticity and memory, decreasing the levels of mitochondrial superoxide in mice increases their longevity.

2. Materials and methods

2.1. SOD-2 transgenic mice

The construction of SOD-2 transgenic mice has been previously described (Ho, Vincent, Dey, Slot, & Crapo, 1998). All mice were housed in Baylor College of Medicine's Transgenic Mouse Facility, compliant with the NIH guide for Care and Use of Laboratory Animals. The facility is kept on a 12-h light-dark cycle, with a regular feeding and cage cleaning schedule. Heterozygote mice expressing human SOD-2, driven by the β -actin promoter, were compared with wild-type mice from the same litter for all experiments.

2.2. Western blot analysis

In order to analyze regional SOD-2 expression in the transgenic mice, the entire hippocampus was dissected fresh on ice from PBS-perfused brains from 14 to 15 mice per group. Dissected tissue from sets of 3–4 mice were combined for homogenization and subjected to Western blot analysis using a rabbit anti-recombinant human SOD-2 antibody (Chang, Kang, Slot, Vincent, & Crapo, 1995), followed by densitometric measurement as previously described (Hanford et al., 2003). The endogenous SOD-1 and catalase levels in hippocampus were determined by Western blots as previously described (Hu et al., 2006). Densitometric analysis of immunoreactivity for each protein was conducted using Scion image software (Scion Corporation). Each experiment was repeated at least three times to ensure validity of the results.

2.3. Detection of mitochondrial O_2^- in primary neuronal culture

Cultures of dissociated hippocampal neurons were isolated from 1 to 2 day postnatal mice as described previously (Wu, Deisseroth, & Tsien, 2001). Neurons at 15–18 days *in vitro* (d.i.v.) were incubated with 300 nM MitoTracker-Green FM (Molecular Probes) for 40 min and then reacted with 5 μ M MitoSOX-Red (Molecular Probes) for 10 min. The treated neurons then were fixed by 4% paraformaldehyde in phosphate-buffered saline and evaluated for mitochondria (MitoTracker-Green FM: Ex λ 490 nm, Em λ 516 nm) and superoxide (MitoSOX-Red: Ex λ 488 nm, Em λ >590 nm) with a Zeiss LSM 510 confocal microscope system (Zeiss, Germany).

2.4. *In vivo* detection of O_2^- using dihydroethidium (DHE)

Dihydroethidium was obtained from Molecular Probes (Eugene, OR). To identify cell-specific superoxide formation in the brain *in vivo*, we utilized DHE as previously described (Hu et al., 2006). Cover slips were mounted with Vectashield H-1200 containing DAPI (Vector Laboratories, Burlingame, CA). Slices were evaluated for ethidium fluorescence (Ex λ 488 nm, Em λ >590 nm) and DAPI (Ex λ 405 nm, Em λ between 420 and 480 nm) with a Zeiss LSM 510 confocal microscope.

2.5. Electrophysiology

Hippocampi from age-matched littermates were removed and 400 μ m slices were prepared using a vibratome. The slices were perfused for 1–2 h with oxygenated artificial cerebrospinal fluid (ACSF) (in mM: 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 $NaHCO_3$, 25 D-glucose, 2 $CaCl_2$, and 1 $MgCl_2$) in an interface tissue slice chamber at 30–32 °C. Basal synaptic transmission (input/output) and PPF were examined in these studies as described in Hu et al. (2006). LTP was induced by one of the following three protocols: one train, three trains (ITI 20 s) or four trains (ITI 5 min) of 100 Hz HFS for 1 s after at least 20 min of stable baseline recordings. fEPSPs were recorded every 20 s and were presented as the average of four individual traces.

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