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17β-Estradiol-mediated increase in Cu/Zn superoxide dismutase expression in the brain: A mechanism to protect neurons from ischemia

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ABSTRACT

A number of studies have demonstrated that 17β-estradiol (E_2) protects the brain from ischemia and yet the mechanism by which this hormone brings about its protective effect is unclear. Interestingly, like E_2, overexpression of the oxidative stress response protein Cu/Zn superoxide dismutase (SOD1), which plays a critical role in regulating reactive oxygen species, also protects the brain from ischemia. Because we previously showed that E_2 treatment of cultured mammary cells increases SOD1 expression, we hypothesized that E_2 might increase SOD1 expression in the brain and that this E_2-mediated increase in SOD1 expression might help to protect the brain from ischemia. We now show that SOD1 is expressed in cortical neurons, that SOD1 expression is increased by exposure of brain slice cultures to E_2, and that the E_2-mediated increase in SOD1 expression is further augmented by exposure of brain slice cultures to increased superoxide levels or oxygen and glucose deprivation. Importantly, when cortical neurons are exposed to increased superoxide levels and markers of protein and DNA damage, nitrotyrosine and 8-oxoguanine, respectively, are measured, both protein and DNA damage are reduced. In fact, E_2 reduces nitrotyrosine and 8-oxoguanine levels in brain slice cultures regardless of whether they have or have not been exposed to increased superoxide levels. Likewise, when brain slice cultures are treated with E_2 and deprived of oxygen and glucose, 8-oxoguanine levels are reduced. Taken together, these studies provide a critical link between E_2 treatment, SOD1 expression, and neuroprotection and help to define a mechanism through which E_2-mediated neuroprotection may be conferred.

1. Introduction

As cells consume oxygen to produce the energy needed to sustain life, they generate reactive oxygen species (ROS), which are involved in a variety of molecular signaling pathways [1]. However, if ROS levels rise and are not effectively dissipated, accumulated ROS can damage resident proteins, lipids, and DNA. It has been suggested that the accumulation of ROS, which include superoxide, hydrogen peroxide, and hydroxyl radical (Fig. 1A), are involved in age-related degeneration and that ROS-mediated damage over time may, in fact, be the cause, not a side effect of aging [2–4].

The brain is the most metabolically active organ in the body. It utilizes 20% of the oxygen consumed and yet accounts for only 2% of the total body mass [5,6]. With this high consumption of oxygen comes increased production of superoxide. It has been suggested that this increased superoxide production, coupled with the relatively low concentration of antioxidant enzymes in the brain, plays a role in age-related neurodegeneration [6,7].

In order to avoid ROS-induced damage to cellular macromolecules, cells rely on a host of proteins to convert ROS to less harmful substances. Cu/Zn superoxide dismutase (SOD1) plays a key role in preventing ROS-induced damage by converting superoxide to hydrogen peroxide, which is then converted to water by catalase and glutathione and thioredoxin peroxidases (Fig. 1A). Dysregulation of any one of these antioxidant enzymes can cause an imbalance in ROS distribution. Thus, it is not surprising that alterations in SOD1 activity have been linked to a number of neurodegenerative diseases including familial amyotrophic lateral sclerosis and Alzheimer's disease [8,9].

An elegant series of studies using middle cerebral artery (MCA) occlusion in rodents as a model of stroke demonstrated that 17β-estradiol (E_2) protects the brain from ischemia [10–12] and that estrogen receptor alpha (ERα) is responsible for mediating this protective effect [13,14]. Interestingly, damage to the cerebral cortex is also significantly reduced in transgenic mice that overexpress SOD1 following MCA occlusion compared to their wild type counterparts [15,16]. Thus, either E_2 or SOD1 overexpression significantly limits ischemia-induced damage in the cerebral cortex.

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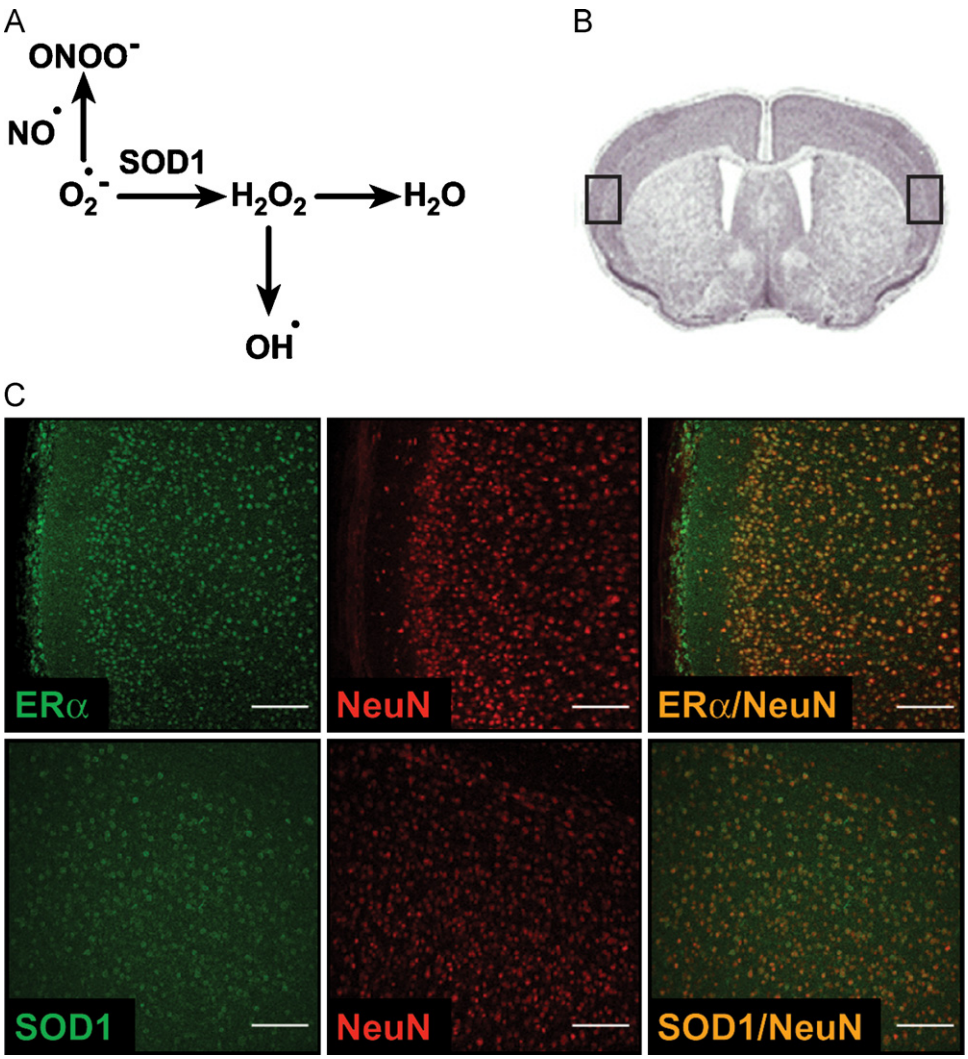

Fig. 1. Expression of ERα and SOD1. (A) During normal cellular metabolism, SOD1 converts superoxide ($O_2^{-\bullet}$) to hydrogen peroxide (H_2O_2) which is then converted to water by catalase and peroxidases. If SOD1 is limiting, superoxide can accumulate and react with nitric oxide ($NO^{\bullet}$) to produce peroxynitrite ($ONOO^-$), which in turn causes nitration of tyrosine residues. If hydrogen peroxide is not effectively eliminated, highly toxic hydroxyl radical ($OH^{\bullet}$) can form. (B) Regions of the cerebral cortex examined in our studies by immunohistochemical analyses are indicated with a box. (C) The expression of ERα, SOD1, and the neuronal marker, NeuN, were examined in brain slice cultures. Scale bars indicate 100 μm.

Previous work from our laboratory demonstrated that E_2 increases SOD1 expression in MCF-7 human breast cancer cells and protects these cells from superoxide-induced protein damage [17]. Thus, we hypothesized that if E_2 had a similar effect in the brain, an E_2-induced increase in SOD1 expression might help to protect the brain from ROS-induced damage. Herein, we demonstrate that E_2 does indeed increase SOD1 expression in the cerebral cortex. Our findings suggest that this E_2-induced increase in SOD1 expression helps to limit protein and DNA damage and thereby protects the brain from ischemia and ROS-induced damage.

2. Materials and methods

2.1. Mice

C57BL/6J mouse colonies (Jackson Laboratory, Bar Harbor, Maine) were maintained on a 12 h light/dark schedule with access to water and food ad libitum. Whole brains were obtained from 5 to 9 day old female pups in accordance with guidelines of the University of Illinois at Urbana-Champaign Institutional Animal Care and Use Committee and Division of Animal Resources.

2.2. Brain slice cultures

Brain slice cultures were prepared essentially as described [18] with modifications. Pups were decapitated and whole brains were quickly removed, mounted, and sectioned with a Leica VT1200 microtome (Leica Microsystems, Nussloch, Germany). 300 μm coronal sections were sliced into chilled slicing solution (1.25 mM NaH_2PO_4, 2.5 mM KCl, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 234 mM sucrose, 11 mM glucose and 26 mM $NaHCO_3$) that had been oxygenated with 95% O_2 and 5% CO_2. Slices were immediately placed on a Millicell sterilized culture plate insert (PICMO3050, Millipore, Billerica, MA) in each well of a chilled six-well plate containing 1 ml of Neurobasal-A medium, 0.5 mM GlutaMAX (12349 and 35050, respectively, Gibco, Carlsbad, CA), antibiotics (gentamicin, penicillin and streptomycin), and 10% charcoal dextran-treated fetal bovine serum (CDFBS) with ethanol or 20 nM E_2. Slices were maintained in a 36 °C incubator in air with 5% CO_2 for 24 h.

2.3. Preparation of brain extracts and Western blot analysis

Each brain slice was combined with 250 μl of RIPA buffer (Thermo Scientific, Rockford, IL) and Protease Inhibitor Cocktail

(Sigma, St. Louis, MO) and homogenized for 10 seconds at low speed with a Pro Homogenizer (ProScientific Inc., Oxford, CT). The buffer was adjusted to 400 mM NaCl with 5 M NaCl, placed on ice, and vortexed every 5 min for 15 min. The extract was spun 14,000 rpm in a 4 °C microfuge. The supernatant was removed, a protein assay was performed, and 15 μg of protein was loaded onto each lane of a denaturing 4–20% gradient gel and fractionated. Proteins were transferred to a nitrocellulose membrane and the blot was probed with the SOD1-specific antibody (sc11407 Santa Cruz Biotechnologies, Santa Cruz, CA).

2.4. Immunohistochemistry

Brain slices were rinsed with PBS, fixed in PBS with 4% formaldehyde for 1 h, washed 3× with PBS containing 0.1% Tween (PBS-T), and incubated in blocking solution (PBS with 0.1% Triton X-100, 3% bovine serum albumin, and 2% normal donkey serum) for 1–2 h. Slices were then incubated in blocking solution with an ERα-, γ-H2AX-, 8-oxoguanine- (1:600, ab31312, 1:400, ab2893, and 1:400, ab64548, respectively, Abcam Inc., Cambridge, MA), SOD1- (1:100, sc11407, Santa Cruz Biotechnologies, Santa Cruz, CA), nitrotyrosine or NeuN- (1:200, 06-284 or 1:600, MAB377, Millipore, Billerica, MS) specific antibody overnight at 4 °C. Slices were washed 3× with PBS-T and incubated with DyLight 488- or 649-conjugated anti-rabbit or anti-mouse IgG, respectively (711-486-152 and 715-496-150, respectively, Jackson ImmunoResearch laboratories Inc., West Grove, PA) for 1 h in the dark, washed 3× with PBS-T, incubated with DAPI nucleic acid stain (1.25 μg/ml, D1306, Molecular Probes, Eugene, OR), washed 3× with PBS-T, and mounted with ProLong Gold antifade reagent (P36930, Invitrogen, Carlsbad, CA). DAPI co-staining was included for each treatment to identify nuclei and ensure that similar numbers of cells were present. Control slices, which had not been exposed to primary antibody, were processed in parallel. Images were obtained with a 40× oil-immersion or 10× objective using the Leica DM 4000 B confocal microscope and Leica TCS SPE system and Application Suite Advanced Fluorescence software (Leica Microsystems, Inc., Bannockburn, IL).

2.5. Oxidative stress induction

For superoxide production, brain slice cultures were prepared as described above. After 24 h, a fresh stock solution of potassium superoxide dissolved in DMSO was prepared as described [19,20]. One microliter of this solution was added to 1 ml of antibiotic-free Neurobasal-A medium with 10% CDFBS and ethanol or E_2 yielding a final concentration of approximately 3.6 μM KO_2 in the experimental wells [21,22]. Control slices were treated with DMSO and processed in parallel. Slice cultures were placed in a 36 °C incubator for 1 h, media was removed, and cultures were incubated with fresh antibiotic-free Neurobasal-A medium with GlutaMAX, antibiotics, 10% CDFBS, and ethanol or 20 nM E_2 for 24 h and then processed for immunohistochemical analysis as described above.

For oxygen glucose deprivation (OGD), brain slice cultures were prepared as described in Section 2.2. After 24 h, inserts containing brain slice cultures were transferred to new plates and incubated in Dulbeccos PBS with calcium and magnesium (DPBS) for 5 min and then the PBS was removed. The washed experimental slices were incubated in DPBS containing ethanol or E_2, and 16.7 mM mannose, which had been deoxygenated with 95% N_2 and 5% CO_2 for 45 min, and were placed in a 36 °C closed anaerobic chamber (ProOx, BioSpherix, Redfield, NJ) with 95% N_2 and 5% CO_2. Control slices, which had not been exposed to OGD, were incubated in DPBS, ethanol or E_2, and 41.75 mM glucose and were maintained in a 36 °C incubator with air and 5% CO_2. After 45 min, the buffer was removed and replaced with fresh Neurobasal-A medium with GlutaMAX, antibiotics, 10% CDFBS, and ethanol or 20 nM E_2 for 24 h and then processed for immunohistochemical analysis.

2.6. Image collection and quantitation

Z-stacks from 3 fields, which contained 50 images per field, were analyzed for each treatment in 3 independent experiments. Detector gain and offset, laser power, and bandwidth for emission collection were kept constant for each treatment in each experiment and adjusted so that images had a full range of pixel intensities (0–255) while minimizing saturation. Image Pro Plus software (Media Cybernetics, Bethesda, MD) was used for quantitative analyses of immunofluorescent images. The perimeter, area, and density/intensity were adjusted so that only specifically stained cells were detected and recorded in a script designed to analyze the individual images in an entire Z-stack. Data were exported into Excel and the mean density/intensity of specifically stained cells in each Z-stack image was quantitated for each treatment. SAS 9.1 Basic Statistics (SAS Institute, Cary, NC) was used for statistical analysis. Data from 3 independent experiments were combined and are presented as the mean ± SEM. One-way analysis of variance (ANOVA) was used and p-values ≤ 0.05 were considered to be statistically significant.

3. Results

While the actions of E_2 on the female reproductive tract have been described in detail [23–25], the mechanisms by which this hormone influences neural cell function are less well understood. A number of studies have demonstrated that occlusion of the MCA causes profound damage to the cerebral cortex [12,13,15,26–28]. However, if a rodent is pretreated with E_2 or if a mouse overexpresses SOD1, the area damaged by MCA occlusion is significantly diminished [12,13,15,26–28].

To understand how E_2 and SOD1 might mediate their neuroprotective effects, we utilized brain slice cultures, which retain many of the structural and organizational features of the intact tissue, allow for manipulation of the cellular environment, and avoid many of the confounding factors involved in using intact animals [18,20]. Brain slice cultures have been widely used to study electrophysiological properties, angiogenesis, dendritic growth, neural cell migration, and the responsiveness of neural cells to various drugs and treatments and provide a valuable in vitro model system that retains many in vivo characteristics [29–34].

3.1. Expression of estrogen receptor α (ERα) and SOD1 in the cerebral cortex

To assess whether our brain slice cultures might serve as an appropriate model system to study the neuroprotective effects of E_2 and SOD1 in the brain, we examined the expression of ERα and SOD1 in the cerebral cortex (Fig. 1B, boxed regions), which is adversely affected by MCA occlusion [12,13,15,26–28]. Since earlier studies had demonstrated that ERα, but not ERβ, confers the neuroprotective effect of E_2 in the rodent brain [13,14], we focused our studies on ERα.

Immunohistochemical analysis of brain slices from wild type mice demonstrated that ERα was expressed in the mouse cerebral cortex and that it was present in the nuclei of neurons as shown by costaining with DAPI (data not shown) and the neuronal marker, NeuN (Fig. 1C). Although modest cytoplasmic staining was observed, SOD1 was highly expressed in the nuclei of neurons in the cerebral cortex. In contrast, no staining was observed when the SOD1 antibody was omitted (data not shown). Thus, both SOD1 and ERα were present in nuclei of the cerebral cortical neurons.

3.2. E_2-induced expression of SOD1 in the brain

Because previous studies have demonstrated that either E_2 treatment or SOD1 overexpression protects the cerebral cortex from ischemia [12,13,15,26–28] and we had shown that E_2 increased SOD1 expression in MCF-7 human mammary cells [17], we determined whether E_2 altered SOD1 expression in the brain. We hypothesized that if E_2 could increase SOD1 expression, this E_2-induced increase in SOD1 expression might help to protect the brain from ischemia.

Brain slice cultures were isolated and treated with ethanol or E_2 for 24 h. As seen in Fig. 2A, SOD1 staining was observed in the absence of E_2, but when brain slice cultures were treated with E_2 for 24 h, SOD1 staining was increased. Compiled data from 3 independent experiments demonstrated that SOD1 expression was significantly increased when brain slice cultures had been treated with E_2.

To examine the effect of E_2 on SOD1 expression in the brain using another independent method, brain slice cultures from 3 individual mice were incubated with ethanol or E_2 for 24 h, whole cell extracts were prepared, and SOD1 levels were monitored by Western blot analysis. E_2 treatment of brain slice cultures increased SOD1 expression in each of the 3 mice. These findings confirmed that E_2 increased expression of SOD1 and demonstrated that our brain slice cultures provided a unique model system to study estrogen responsiveness.

3.3. Superoxide and E_2-induced expression of SOD1 in the cerebral cortex

Because SOD1 is required to convert superoxide to hydrogen peroxide and plays such a critical role in regulating ROS levels (Fig. 1A), we determined whether increased substrate, superoxide, would influence SOD1 expression in the cerebral cortex. When brain slice cultures were exposed to increased superoxide levels, which were produced by addition of potassium superoxide (KO_2) to the medium, SOD1 expression was enhanced (Fig. 2A). Exposure of the brain slice cultures to both E_2 and KO_2 further increased SOD1 expression. Quantitative analysis of data from 3 independent experiments demonstrated that there were significant increases in SOD1 expression in response to E_2 and KO_2 individually, but that combined E_2 and KO_2 treatment more effectively increased SOD1 expression. Thus, our brain slice cultures not only expressed ERα and SOD1, but also responded to E_2 treatment and/or elevated superoxide levels with increased SOD1 expression.

3.4. E_2-mediated decrease in protein damage in the cerebral cortex

SOD1 is an essential free radical scavenger of superoxide and is the first line of defense in regulating ROS [35]. If SOD1 is ineffective in dissipating superoxide, accumulated superoxide anions can react with nitric oxide to produce peroxynitrite (Fig. 1A), which in turn causes nitration of tyrosine residues and alteration in protein structure and/or function [36]. Thus, measuring nitrotyrosine levels is a valuable indicator of SOD1 expression and activity. To determine whether the E_2-mediated increase in SOD1 expression helped to limit tyrosine nitration, brain slice cultures were treated with ethanol or E_2 in the absence or presence of KO_2. As seen in Fig. 2B, nitrotyrosine levels were significantly reduced when the brain slice cultures were exposed to E_2 and, although exposure to KO_2 increased protein nitration, E_2 significantly reduced the KO_2-mediated increase in nitrotyrosine levels. Because SOD1 is responsible for dismutating the majority of superoxide produced during oxidative stress [37], the E_2-mediated increase in SOD1 expression could play a vital role in limiting ischemia-induced protein damage.

3.5. E_2-mediated reduction in DNA damage

In addition to damaging proteins, ROS such as peroxynitrite and hydroxyl radical can damage DNA [38]. Guanine is particularly susceptible to oxidation and is the most common target of oxidative DNA damage. 8-Oxoguanine (8-oxoG) mispairs with adenine and results in a guanine to thymine transversion and perturbation in DNA conformation [39]. 8-oxoG levels were examined after exposure of brain slice cultures to ethanol or E_2 in the absence or in the presence of KO_2. No significant change was observed in 8-oxoG levels after brain slice cultures had been treated with E_2 for 24 h (Fig. 2C). Although a KO_2-induced increase in 8-oxoG levels was observed in the absence of hormone, damage was significantly attenuated when the brain slice cultures had been treated with E_2 and then exposed to KO_2. These findings provide evidence that E_2 limits damage in the cerebral cortex and identify a mechanism by which E_2 may mediate its protective effect.

Oxidative stress can also cause double-stranded DNA breaks and lead to genomic instability. The histone H2A variant, H2AX, recruits DNA repair proteins to these double-stranded breaks [40,41]. Although no significant changes were observed in γ-H2AX levels in the presence of E_2 and/or KO_2 (Fig. 2D), it seems plausible that the active recruitment of DNA repair proteins to H2AX and subsequent repair during the 24 h recovery period could mask changes in γ-H2AX levels that might have occurred in response to E_2 or KO_2. Such rapid repair of these lesions has been reported [42].

3.6. Oxygen glucose deprivation (OGD) and cellular damage

To more closely simulate ischemia, brain slice cultures were treated with ethanol or E_2 for 24 h, deprived of oxygen and glucose for 45 min, and allowed to recover under normoxic conditions for 24 h in the absence or presence of E_2. Immunohistochemical analysis indicated that ERα levels were significantly increased when the brain slice cultures were exposed to E_2 and OGD, but not when exposed to E_2 or OGD alone (Fig. 3A) suggesting that increased ERα levels may be particularly important in protecting the brain from ischemia when the cells are exposed to both E_2 and OGD.

When brain slice cultures were exposed to either E_2 or OGD, an increase in SOD1 expression was observed (Fig. 3B). SOD1 expression was further enhanced when cells were treated with E_2 and then subjected to OGD. These findings are consistent with those shown in Fig. 2A and demonstrate that two independent methods of generating oxidative stress have similar effects on SOD1 expression and that either elevated superoxide levels or OGD enhance SOD1 expression but that E_2 further augments its expression.

To assess whether DNA damage occurred when the brain slice cultures were exposed to OGD and whether E_2 influenced the extent of DNA damage, 8-oxoG levels were examined in brain slice cultures that had been treated with ethanol or E_2, subjected to OGD or maintained in glucose-containing medium and a normoxic environment, and allowed to recover for 24 h in the absence or presence of E_2. Interestingly, although OGD increased 8-oxoG levels, E_2 significantly reduced this OGD-mediated increase (Fig. 3C). These findings provide additional evidence that E_2 is protective in the cerebral cortex and help to define mechanisms by which ROS-induced DNA damage might be limited.

4. Discussion

A number of investigators have demonstrated that treatment of wild type rodents with E_2 or overexpression of SOD1

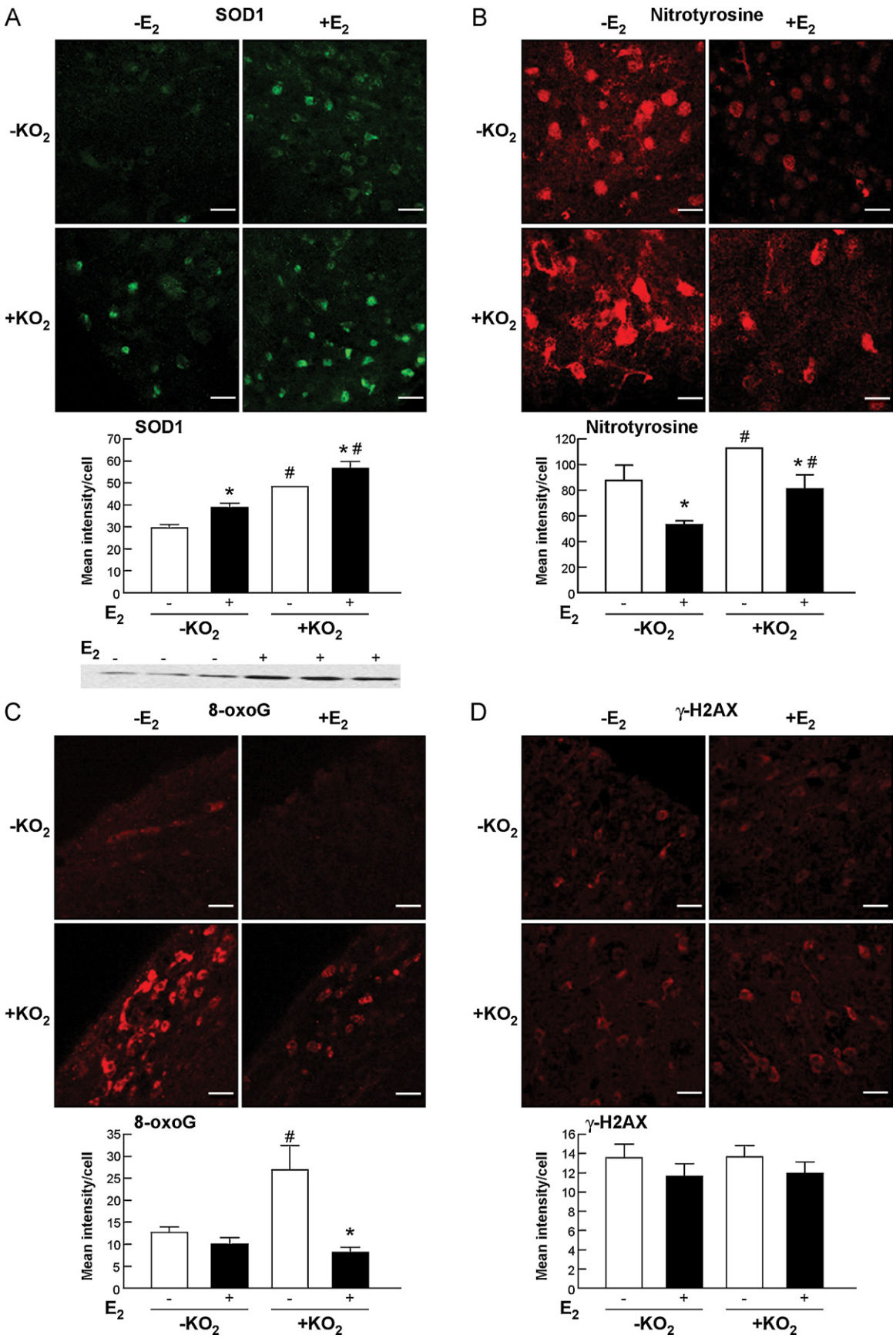

Fig. 2. Effect of E_2 and superoxide in the cerebral cortex. Brain slice cultures were treated with ethanol or 20 nM E_2 for 24 h, exposed to DMSO or KO_2 for 1 h to generate increased superoxide levels, allowed to recover for 24 h, and then subjected to immunohistochemical analysis with an (A) SOD1-, (B) nitrotyrosine-, (C) 8-oxoG-, or (D) γ-H2AX-specific antibody. Scale bars indicate 25 μm. Data from 3 independent experiments were combined and analyzed by ANOVA. A significant difference in response to E_2 ($^{*}p \leq 0.05$) or KO_2 ($^{\#}p \leq 0.05$) is indicated. (A) SOD1 expression was also analyzed with Western blot analysis using brain slice cultures from 3 individual mice that had not (−) or had (+) been exposed to E_2.

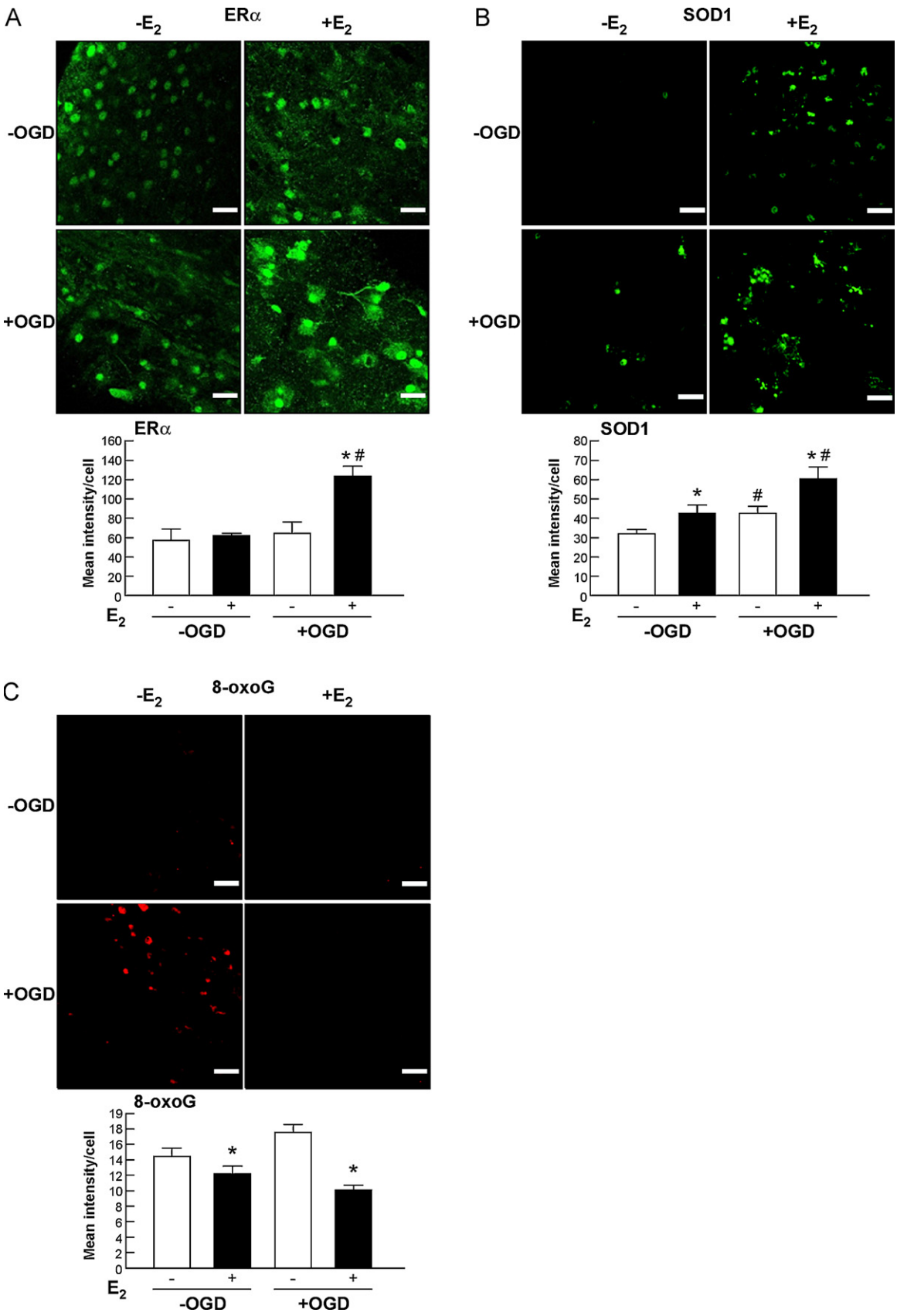

Fig. 3. Effect of E_2 and OGD in the cerebral cortex. Brain slice cultures were treated with ethanol or 20 nM E_2 for 24 h with or without OGD for 45 min to mimic ischemia, and then allowed to recover for 24 h. Brain slice cultures were subjected to immunohistochemistry with an (A) ERα-, (B), SOD1- or (C), or 8-oxoG-specific antibody. Data from 3 independent experiments were combined and analyzed by ANOVA. A significant difference in response to E_2 ($^{*}p \leq 0.05$) or OGD ($^{\#}p \leq 0.05$) is indicated. Scale bars indicate 25 μm.

in transgenic mice protects the brain from an ischemic event [12,13,15,26–28]. We now provide evidence for a link between E_2 treatment, SOD1 expression, and neuroprotection. Our studies demonstrate that E_2 increases SOD1 expression in the cerebral cortex of brain slice cultures and suggest that this E_2-induced increase in SOD1 expression helps to attenuate protein and DNA damage that occurs in response to increased superoxide levels and OGD treatment.

4.1. SOD1-induced neuroprotection

Compelling accumulated evidence has demonstrated that SOD1 protects the brain from ischemia-induced damage [15,16,43,44]. Transgenic mice that overexpress SOD1 have less extensive brain damage, decreased cytochrome C release into the cytoplasm, and diminished DNA fragmentation after ischemia than their wild type counterparts [15,16,43–45]. Furthermore, the fact that SOD1-deficient mice have more extensive brain injury after local and global artery occlusion than wild type mice confirms the critical role of SOD1 in protecting the brain from an ischemic insult [28]. While an earlier study showed that hippocampal injury is decreased in female mice or in mice that overexpress SOD1 [46], our studies are the first to show a direct link between E_2 treatment and increased SOD1 expression.

4.2. ROS-induced damage to cellular macromolecules

Regardless of whether brain slice cultures had or had not been exposed to increased superoxide levels, tyrosine nitration, which is directly related to SOD1 levels, was decreased by E_2 treatment. Even when tyrosine nitration was increased by exposure to elevated superoxide levels, E_2 was still effective in reducing this damage. Because nitration of tyrosines can have deleterious effects on protein structure and function, the E_2-mediated increase in SOD1 expression in the brain could limit peroxynitrite and nitrotyrosine formation and play a significant role in E_2-induced neuroprotection. In fact, SOD1 dysfunction has been noted in a number of neurological diseases and disorders [47,48]. The fact that nitrotyrosine residues were detected in control brain slice cultures that had not been exposed to E_2 or oxidative stress illustrates that these cells tolerate some residual protein damage and that oxidative stress proteins may be continually required to maintain protein structure and function.

ROS-induced DNA damage occurs most frequently at guanine residues and results in the production of 8-oxoG, the most common DNA lesion [49]. It has been estimated that 16,000 8-oxoG adducts are produced per cell each day [49]. If not repaired, these modified guanines can accumulate over time and increase the risk of age-related disease [4]. Because SOD1 is the first line of defense in regulating cellular ROS, the E_2-mediated increase in SOD1 expression could provide a mechanism by which ROS-induced DNA damage in the cerebral cortex may be attenuated. In fact, Inoue et al. [50] have proposed that SOD1 is instrumental in limiting DNA damage and functions as a guardian of the genome. Our studies suggest that the E_2-induced increase in SOD1 expression could be instrumental in maintaining genomic integrity.

4.3. Effects of E_2 in the brain

Interestingly, when brain slice cultures were treated with E_2 and exposed to OGD, there was an increase in ERα expression in the cytoplasm as well as the nucleus (Fig. 3A). The localization of ERα in the cytoplasm after OGD treatment suggests that, in addition to its genomic effects, ERα may be involved in extranuclear functions when E_2-treated neurons are subjected to oxidative stress. A similar rise in ERα mRNA levels has been reported when mice are ovariectomized, immediately treated with E_2, and subjected to MCA occlusion, but not when E_2 treatment is delayed [11]. Likewise, when brain slice cultures were exposed to E_2 and OGD, cytoplasmic SOD1 staining was enhanced (Fig. 3B). Since cytoplasmic SOD1 has been implicated in inhibiting the release of cytochrome c after ischemia/reperfusion injury [45], the increased cytoplasmic ERα and SOD1 levels we observed when brain slice cultures were treated with E_2 and exposed to OGD could help to sustain cell viability in the cerebral cortex.

4.4. Role of other proteins in E_2-induced neuroprotection

Although our studies focused on the E_2-induced expression of SOD1, it seems certain that other proteins are involved in the E_2-mediated decline in protein and DNA damage. SOD2, which is localized in the mitochondria, also dismutates superoxide and is responsible for reducing superoxide produced during the course of normal cellular metabolism [37]. However, since SOD1 is responsible for converting the majority (75%) of cellular superoxide to hydrogen peroxide and for decreasing superoxide levels during oxidative stress, it seems likely that SOD1 plays a more dominant role in decreasing protein and DNA damage caused by ischemia/reperfusion injury.

Previous studies have suggested that Bcl-2 is involved in E_2-mediated neuroprotection [10,51]. While increased Bcl-2 may be important in decreasing cell death, simply limiting apoptosis in cells with extensive protein and DNA damage would not be useful. However, if the increased expression of proteins involved in oxidative stress response and DNA repair was coupled with increased Bcl-2 expression, together, these proteins could help to reduce protein and DNA damage, maintain cell viability, and reduce stroke-induced cellular damage.

5. Conclusions

Our studies have provided a link between E_2 treatment and increased SOD1 expression in the cerebral cortex. Because overexpression of SOD1 protects the brain from ischemia [15,16,43,44] and decreased SOD1 expression is associated with increased ischemia-induced damage [28], we believe that the E_2-induced expression of SOD1 may help to protect the brain from ischemia-induced injury by decreasing nitration of cellular proteins and limiting ROS-induced DNA damage.

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